MAIN 96: An interactive software for density modifications, model building, structure refinement and analysis

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Abstract

MAIN is a molecular graphics program, which provides outstanding graphical presentation of molecular and map images, designed to deal interactively with complex tasks of macromolecular crystallography. With MAIN it is possible to perform density modifications, model building, structure refinement and various types of molecular structure analysis. The prompt availability of various analytical tools and immediate visualisation of molecular and map objects allows a user to efficiently reduce number errors. It is a single executable program that intensively uses computer hardware resources, with a general premise that combined use of computational methods and molecular graphics significantly simplifies and speeds up a structure determination process.

Introduction

The computational part of macromolecular structure determination can be divided into the following tasks:

- Diffraction data processing and evaluation,
- Initial phase set construction (by molecular or isomorphous replacement approaches),
- Electron density map modifications,
- Electron density map interpretation or model building,
- Structure refinement and
- Structure analysis.

There are a variety of programs and suites that more or less sophisticatedly address specialized applications within each particular group. MAIN is oriented to interactive work. It includes tools that cover the tasks associated with the last four items. Current software development has a general tendency for a software integration into self-consistent environments, which are becoming more and more complex, but still open to other programs. One extreme course of software integration is the CCP-4[1] project, where software contributions from many members of the crystallographic community are put together and distributed. Tools are donated and developed on a one-by-one basis. The communication between various executable files runs through standardized files. MAIN goes against this development trend, it is a single executable backbone which holds all data in computer memory during a run. Data are stored to disk for backup reasons, in order to enable communication with other programs and in order to save results at the end of a working session. During a run reading and writing files is reduced to a minimum.

The development of MAIN is going into its eleventh year[2]. MAIN was first a molecular graphics program that turned into molecular modeling program sometime after my arrival at Martinsried in 1988 and then tools to support crystal structure determination were gradually added to the program. The first crystallographically complex task was to generate solvent molecules around a protein, with checks including 2Fobs - Fcalc and Fobs - Fcalc difference density map checks, verifying hydrogen bond donor and acceptor environment and symmetry overlaps. The slightly modified routine is still included in MAIN distribution. In 1990, density averaging followed by solvent flattening was incorporated. MAIN 91 was still running on an Evans & Sutherland PS300 and VAX VMS computer [2]. The years after, ports to ESV, SGI, HP and lately DEC workstations followed. Introduction of Fast Fourier Transformation (FFT) routines in 1995 encircled the density modification routines and gave a basis for interactive map calculations and structure refinement.

Program description

MAIN is a research project in which I intend to encompass the essential methods necessary for a molecular structure determination. MAIN is an open software, a kind of backbone, which allows and encourages a user to program his own macros and interact with a variety of other programs as well.

MAIN is written to support and replace manual work and to do more work than other programs (to do more map calculations, more model building, more structure refinement and more structure analysis), because MAIN fulfills the basic principle of interactivity: you can ask questions and obtain the answers before the questions are forgotten. The ability to perform quite a number of tasks and analyse their results in a short time introduces a novel understanding of a macromoelcular structure determination process.

Molecular and map images

MAIN can display elementary molecular colored images (bonds as lines, atom crosses, ball and stick models, Connolly surfaces [3], atom and residue names, ...). In addition interatomic distance, pair lists and hydrogen bonds as well as forces acting on atoms can be displayed. Consistency of atomic and residue records with topology files is not at all necessary. To display a molecule, its atoms should have a name and position in space. Maps can be displayed as wire frame objects and contoured in X, Y and Z layers simultaneously or separately or as polygonal areas. It is also possible to present values of density at grid points as numbers displayed at their grid point position.

Colors can be selected through the MAIN internal color tables using dials. A user can, however, create his own color palettes. Background color as well as contrast of image to the background can be changed. Molecular images and map can be converted into PostScript files, with the exeption of polygonal objects.

Model building

Model building in the MAIN environment is composed of creation or modification of topology of molecules (connectivities and atom and residue records) and their placement into desired positions and conformations. The topology editor includes tools for renumbering residues, rearranging segments and renaming any possible string-based records including the default definitions of the periodic system of elements, which is a basis for scattering factors data. Positional changes are either manual or minimizer driven.

Tools for manual interaction with conformation and position of the molecular model are certainly one of the highlights of MAIN. Geometry changes can be imposed on any structure, presuming that its atoms are displayed and possible connectivities (covalent bonds) between them exist. Individual or groups of atoms can be moved around based on absolute or relative descriptors. Absolute descriptors are translation vectors and rotation matrices. Relative descriptors refer a rotation or translation of a group of atoms to some picked atoms. So it is possible to impose rotations about bonds (so-called chain rotations), to change bonding angles and to move groups of atoms along a line connecting two atoms. Any of the operators can be combined in any order, so you can for example impose rotation and translation on an inhibitor molecule and enable a number of chain rotations simultaneously. It is also possible to change the conformation of a molecule by rotations about the same bond in both directions simultaneously. Monitoring a practically unlimited number of distances, angles and dihedral angles, including positioning of a residue within Ramachandran space, is also possible.

A secondary structure table includes all basic secondary structure elements and such exotic things as polyproline and collagen folds. Type I, II, III and gamma turns and their inverses are available too. The folding elements can be assigned in forward or backward directions to any size chain segment. A user can browse through them by clicking until a reasonable starting point for electron density interpretation is found. These functions can be called also when manually driven positional changes of the model are activated.

A user can get the most out of MAIN when manually driven geometry changes of a model are combined with energy minimization procedures.

Energy calculation and minimization

Energy calculations are based on topology libraries and force field parameters. For proteins the Engh & Huber [4] parameter set is generally used. Nucleic acid files are available as well. In general these files are ASCII and can be read and written by the program. With MAIN it is possible to interchange information between molecular topology, geometry and energy term lists and topology library records. This means that a user can create his own residue entries. The simplest way of doing it (if you are an X-PLOR user) is to read in native X-PLOR [5] topology and parameter files and a corresponding atom coordinates file, define the bonding lists and atom types (classes) and re-create the new topology entries in MAIN format. This allows you not only to perform energy cal-

culations, but also later to edit molecular topology, for which X-PLOR topology files are not sufficient.

Besides standard bonding (bonds, angles, dihedrals and impropers) and nonbonding (VdW and electrostatics) energy terms, density correlation, dihedral, NCS, pair and hydrogen bonding constraints can also be applied. The density correlation term pulls atoms against peaks of curvature of local density; NCS constraints pull different molecular models against each other. The pair constraints pull atoms against each other towards a specified distance with a harmonic force, and hydrogen bonding constraints are treated similarly. Dihedral constraints try to enforce certain conformations.

A density correlation term should be used throughout the model building sessions. It helps to regularize the geometry of the model with density constraints. Convergence is limited by the smoothness of the density gradients. Use of a very highly scaled density term has quite long convergence radii, sometimes exceeding 2Å. After a density fit, when it results in severe geometry violations, the scale should be brought down. This does not always work, so MAIN also has distance constraint means to pull the model across larger distances. Since constraint lists are editable (they can be calculated and terms added or removed from them on an individual basis) they are a flexible way of enforcing a desired molecular conformation or position. Hydrogen bonds are very useful for regularizing large folding patterns, as are β sheets. Use of pair constraints is more general, the most distinguished application feature of pair constraints being the use of anchors. A user can anchor atoms to their current positions or place the anchors somewhere else, for example at peaks of electron density; acctually anchor positions can be optimized in electron density with a help of a minimizer. Using anchors, energy minimization of the model will allow you to shift parts of the structure without severely distorting their geometry over large distances.

MAIN uses a conjugate gradient minimizer. When no structure factor calculation is included in a minimization procedure, then essentially any size of macromolecule can be minimized in a short time on a moderate workstation. Atoms can be kicked (displayed randomly in X, Y and Z coordinate from their current positions). Kicking is a computationally cheap way of getting out of local energy minima - it helps to reduce a model bias as well. Larger shifts should, however, be carried out manually.

When using the MAIN toolchest, there is no real need for database support. You can always begin with a relatively good starting conformation based on some secondary structure element and then remodel it with the help of energy calculations.

Structure factor manipulations

Structure factor manipulations encompass exchange of information between Fcalc, Fobs and Fwork structure factor sets. Fcalc and Fobs data can be read, including their weights and sigma values. The Fcalc obtains information from the last FFT calculation, and Fwork is the structure factor set that builds a map. Sigma weighting can be applied. Reflections can be manipulated in various subsets, since a logical command parser allows selective addressing of various structure factors on a variety of criteria from their amplitudes, position in reciprocal space, resolution, their r-values, ... to random selections, which allow also R-free calculations. Any selection of Fcalc can be scaled to Fobs to yield an R-value. Scaling is done in shells. Each shell has an individual linear factor, and all of them have an overall B-factor. The number of shells has an effect on maps, atomic positions, B-values and also crystallographic R-value.

Anomalous scattering is not supported yet. Friedel symmetry is used throughout the program.

It is recommended that structure factor records be used instead of electron density maps for storage of phase information and data exchange between various programs. It is significantly faster to perform an FFT calculation than to read in a huge electron density map, and in addition, disk space can be saved.

Electron density generation is based on the five Gaussian forms. Some default values for atomic structure factors are included in the code already. A user is warned about missing scattering parameters.

Map manipulations

Maps are 3-dimensional grid point lattices. Each map has its own unit cell data, grids per unit cell and box (the map origin and the last point in grid coordinates). The lattices are arrays of real*4, character*1, integer*4 or complex*8 numbers. The real and character maps present density, enveloped and empty regions. The value of a grid point tells the program which kind of point it is. The integer maps are used as connectivity tables, and complex maps are input to and output from the FFT routines.

Map manipulation is an important and strongly supported topic within the MAIN environment. The flexibility of the MAIN command parser allows the exchange of map information between computer memory and peripherial devices (disks) to be substantially reduced, which then speeds up map transformations - an important issue when a density modification procedure is invoked during an interactive session.

For efficient and fast work at least two unit cells should

be kept in computer memory. Dealing with complete unit cells is recommended since the use of P1 translations allow maps to be expanded to regions anywhere in space.

Molecular envelope editor

When performing a density modification procedure, its basis is a molecular envelope, which demarcates a solvent region from a macromolecular structure. The envelope is also a map. What makes a grid point in a map a part of an envelope is its value (9999.0). MAIN can calculate envelopes on the basis of statistical means and with the help of interactive display.

Statistical means are based on real- and reciprocal-space calculations of the scoring map. According to the histogram of the scoring map, parts of the map are accepted as molecular regions, and the rest is treated as solvent. A variety of weighting schemes (linear, Gaussian, squared) as well as root mean square (RMS) deviation of the local map or its variance can be applied during a score map creation. Real-space calculations are much more time consuming than the reciprocal-space calculations in which the convolution of a weighting scheme and the underlying density are calculated as a product of their fourier series coefficients. A lot of these has been covered in already published works of Wang, Leslie, Abrahams [6,7,8] and others, although a skilled user can discover and create as yet unknown variances of an envelope generation. A possibility is also a map skeletonization.

Interactive molecular envelope definition can be based on a statistical approach, although the ways to modify an envelope go through atom records. The simplest molecular envelope construction is based on a molecular model. It is sometimes quite easy to modify an envelope by adding α helices or β strands to an existing molecular model. When nothing is available, the best approach is to generate a map skeleton (usually a matter of seconds), display it and then edit its topology so that a user can finally select desired parts of a density map. Editing topology means breaking some connectivities and creating new ones.

The skeletonization procedure as incorporated in MAIN is based on Swanson's idea [9] of searching for local extremes and finding saddle points between them. The difference in implementation is that the procedure does not include any sorting procedures. The procedure involves three passes through a map. In the first pass each grid point within a specified density range points to its highest neighbor. The extreme is a point which does not point to any neighbor, because it has the highest density value among its neighbors. The second pass traverses from extreme points in the reverse direction of the pointers to demarcate the grid points pointing to a local extreme. The third pass finds the border points between various extreme hills. The point with the highest density value is a saddle point. Extreme and saddle points are transformed into atoms and connected with covalent bonds, which can be selected later according to the size of a network and displayed as standard molecular images.

When density averaging is planned, your particle includes no spherical symmetry, and no molecular model is available, then using a skeleton is probably a good idea. First choose an asymmetric unit exploiting the routines generating symmetry mates of the selected skeleton atoms, and then try to break it into individual parts - all through interactive visual control of the skeleton.

When an envelope is created, it can be expanded or reduced, lonely clouds of envelope points can be removed and cavities filled. MAIN is by no means a program that tries to limit you to use of a single procedure only. An experienced user can combine all of them simultaneously.

Solvent flattening

The unchosen part of the density remains after a unit cell generation empty (-9999). This region can be simply flattened [6] or flipped [8], shifted or scaled or modified however the user desires.

Map averaging

Map averaging was the first density manipulation procedure written in MAIN. Human and rat cathepsin B [10] were the first molecules averaged. At that time we also tackled the riboflavin synthase monoclinic form, which saw daylight in a publication last year [11]. It was at the time a giant project (50 000 atoms in an asymmetric unit and 30 molecules per asymmetric unit). Density averaging with phase extension took about half of year on a dedicated MicroVAX. Nowadays, a similar averaging procedure of a proteosome of a comparable size to the riboflavin synthase takes about a day.

Parameters for geometric transformations between equivalent density regions can be obtained by superposition of equivalent molecules by RMS fit procedures, from Patterson maps and from real-space search procedures either done with MAIN or a program as AMoRe [12]. MAIN allows localized map superpositions to be optimized by maximizing the correlation between the background map and rotated and translated points of the superimposed map.

Averaging can be performed between any number of

equivalent regions and any number of different crystal forms and for any possible combination. A requirement is that equivalent regions are contiguous areas in space.

Since unit cell generation maps each grid point to its symmetry-equivalent points by applying the symmetry operators, a user does not have to worry about a particular asymmetric unit definition.

Structure refinement

MAIN uses a so-called **difference density** method, which resembles somewhat the Diamond procedure [13]. However, the positional derivatives are not based directly on a volume overlap, but they are calculated from the curvature of a difference map. The map curvature is approximated with a second-order polynomial or Gaussian function. The simplifications of the density term derivatives procedure are actually the ones that make the whole process interactive on a medium-range workstation, with no need to update the structure factors after a small shift of the model. The density derivatives obtained are part of the force field. They, together with geometry constraints (bonding and nonbonding terms), guide the model against a local minima. There is no difference in refinement against any kind of map - the procedure searches for maximal overlap of a model with the background map. This approach allows global as well as local treatment of the model. When refining a structure globally, the whole structure is energetically minimized against a difference density map for a specified number of steps, the map is updated afterwards with the newly calculated structure factors, and the calculation enters the next cycle.

Non-Crystallographic-Symmetry (NCS) constraints can be imposed between various molecules, also when they arise from different crystal forms. To my knowledge the structure of human procathepsin B was the first reported case of a macromolecular structure in two crystal forms refined simultaneously [14]. NCS constraints can be flagged. This allows a single parameter or any combination of them to be fixed. For example fixing a rotational angle in polar coordinates ($\kappa = 180.0$) can fix a perfect two fold symmetry, while the system is still allowed to move and the direction of rotational axes can be adjusted.

Local refinement is usually part of a model building procedure, where a user fits the model against a density map interactively with extensive use of energy minimization.

The user is advised to randomly interchange local and global model refinement. It should be borne in mind that the whole model should be changed to get away from the model bias of a previous structure. Including and removing parts of a structure from a phase calculation will not affect the phases much.

The **partial structure refinement** procedure uses the information from the current model as well as the information from an underlying background map for structure factor calculation. The idea and derivation are similar to a previously described procedure [15]. The background map can be based on phases from isomorphous replacement, a density modification procedure or from a model based (2Fobs - Fcalc) map. In a region near the molecular model, the background map is merged with the Fcalc map of the model. The traversal through reciprocal space is necessary in order to adjust atomic B-values and to merge maps with the same resolution-dependent truncation error and histograms. Besides it is quite fast. The molecular envelope designates the density region or the molecule where density is pulled along into next cycle. The regions outside can be flattened or flipped. From thus obtained structure factors, two maps are being calculated, the new background map and the new Fobs - Fcalc map. The new difference map is used to guide the model against a better position. This model density is then merged with the background map and starts a new cycle.

MAIN is open to cooperate with other refinement programs, since it is possible during refinement to call a macro which does the structure factor and derivatives map calculations. Macro is a set of MAIN commands which can also call external shell scripts.

Structure analysis

During a model building session a user should often invoke the structure analysis routines. It can save a lot of time and point to the wrongly built spots of a structure. When an asymmetric unit contains more than a single molecule, it is always advisable at least to superimpose images of all other molecules on the current working model.

The simplest structure analysis is to keep images of related superimposed molecules in the background of a currently built model. Turning their images on and off resolves many ambiguities.

The Ramachandran plot monitor enables an inexperienced user to move a polypeptide chain conformation into the allowed regions of a Ramachandran plot while rotating the model about ϕ and ψ angles and monitoring their position in the Ramachandran diagram.

The most flexible structure analysis is based on energy calculations. A user first turns on and off the desired energy terms, calls the interaction energy calculation between each chosen atom and its neighbors.

The atomic interaction energies are averaged within each residue. Then the residue average, RMS, minimal or maximal value can be used to color and display the chain of atoms in colors ranging from blue to yellow. The yellow parts have the highest energy and should be therefore checked first. This way it is possible to color code density correlation, bonding term distortion and also the energy of nonbonding interactions. Usage of nonbonding terms only can pop out wrong orientations of side chains and also bad main chain contacts. The energy-based structure analysis is not statistically based and is therefore not limited to polypeptides only. It can be applied to any kind of molecular topology.

Similarly as interaction energy, atomic B-values can be analyzed and their analysis presented. The most interesting part of B-value analysis is the records of RMS deviations within each residue.

One of the important issues in a structure analysis is the quality of force field parameters. With MAIN it is possible to perform the basic statistical analysis of a used force field (RMS and average deviations from target values) and its consistency with the refined structure. Severe shifts between target and average values indicate that these force field target values should probably be replaced with others or split into more values by introducing novel atom type (class) definitions. These analyses give the overall error of all target values, numbers which are usually used to describe the quality of a structural model.

User interface

Interaction between a user and MAIN is through two interactive device-driven windows (the menu window named 'strikes' and the MAIN image window) and through the shell window, where command sentences are read in. The largest window is the image window. It is the most important window in MAIN, therefore quite some care has been directed to its clarity and input from interactive devices (mouse and dial box) and update of the image. The procedures are optimized in order to achieve the best performace (smooth rotations of vectorized images) and good 3-D objects perception. (Work with Crystal Eyes stereo on a Silicon Graphics workstation does not result in headaches.)

When running MAIN, a user should get familiar with the menus. Menus are organized in pages, so it is possible to scroll through them. Ten pages are supported. Each page is composed of a variety of menu blocks. Each menu
block has its own color and document file and can be displayed on any page. Several menu blocks are encoded into the MAIN source and the rest can be loaded afterwards (by issuing the <>cmds/load_depp_page command). Each menu block consists of menu items. Each item executes a command, which quite often calls a command macro. So mastering a text editor is essential for efficient macro manipulation. Less advanced users are still forced to adapt the macros by inserting the corresponding segment names.

Menu block items, menu block composition and menu page setup are programmable, so an experienced user may wish to enhance the number of commands and macros invoked with a single mouse click. This is probably the only way to deal with more complex cases, when for example a unit cell is composed of several identical molecules in similar spatial arrangement or when they even appear in different crystal cells and a user wants to edit only one of them by exchanging some local fragment conformation between different molecules and proliferate the modified regions into other molecules.

The clicking of menu items parses commands to the MAIN command sentence parser. Command sentence interpretation is the basic running level of MAIN. Commands can call macros and macros other macros up to ten layers deep. String, real and integer value parameters can be transferred and exchanged between macros through variables. Loops based on simple IF commands can be built as well. Complete command input is stored into the 'input.cop' file, so that a user should never be afraid of a computer crash or shutdown, because complete sessions can be retrieved from this file.

The idea of clicking is the following: first atoms as arguments parsed to functions are clicked and then menu items. This way the same argument can be used for more functions, and in the case of an error, the argument list can be corrected. When it is ready, a single click triggers a function. An aditional cancel or execute click is not necessary. A user can select pickable atoms.

Most geometry changes can be undone. But be careful, it is only a single-step coordinate retrieval.

Compatibility with other programs

MAIN is a program with broad applicability but also compatible with a variety of programs. This is the reason why all MAIN files are ASCII and thereby readable by a human eye. MAIN and X-PLOR are completely compatible in terms of ASCII files (atomic coordinates files, topology and force field parameter libraries, reflection files, electron density maps). There are some programs where the exchange of atomic coordinate files is supported (CHARMM, GROMOS, DISCOVER, EREF, GAUSSIAN and some others). Electron density maps can be read and written also in

PROTEIN [16] format and in CCP4 ASCII format files interfaced to CCP4 through the 'mapexchange' program.

Technical data

MAIN is mostly written in FORTRAN 77. It can also be compiled with FORTRAN 90. Some of the graphics and the X-window interface are written in C. The source includes approximately 120 000 lines. It runs on Silicon Graphics workstations with GL graphics (OpenGL release is planned soon), DEC Unix ALPHAS with OpenGL graphics and on Hewlett Packard and Evans & Sutherland V workstations with PHIGS. Releases without graphics are available also for DEC VMS platforms.

An anonymous FTP server (stef.ijs.si) provides documentation, utility files, demos and demonstration executable files. Information about licensing is available there too. Html based documentation provided on the above specified WWW server is underway.

Summary and Conclusions

MAIN addresses electron density map modifications, density map interpretation with model building, structure refinement and structure analysis. There are two major premises of MAIN development: It tries to cover as many topics as possible, and it is designed to perform these jobs interactively, to retrieve and present results within an interactive time limit. MAIN is oriented to present results as 3-dimensional color images rotatable on the screen of a workstation. MAIN offers some unique tools such as simultaneous structure refinement of macromolecules with NCS between various crystal forms, soft group restraints for atomic B values, partial structure refinement, molecular envelope editor, topology libraries editor, Ramachandran plot monitor, color coded energy analysis of structure distortion etc., Its major advantage, however, is that you can apply all these tools immediately, one after another in any order with no need to quit a session. That means that the classical sequence of a model building session followed by a structure refinement cycle with new map calculations and, at the end, the final refined structure analysis can be done within a couple of minutes, invoked by clicking menu items. For example structure analysis immediately shows in a color-coded way where a model is geometrically distorted - so go there immediately and build it correctly.

MAIN is a CPU-intensive program, it occupies as much memory as you can provide and it works nicely with a 3-D graphic board of medium performance. The normal MAIN version behaves well on an Indigo 2 R4400 200MHz with Extreme graphics board and 96Mb or an equivalent, when dealing with a moderate-size protein (up to 800 residues in the asymmetric unit), about 100k reflections, and unit cells which do not include more than 3M grid points. It does not mean that you cannot handle larger structures as batch jobs as, for example, averaging with phase extension of the proteosome [17]; however with the increasing power of workstations even such structures could be dealt with interactively in the foreseeable future.

Acknowledgments

Essentially R. Huber's entire group of friends and colleagues in Martinsried is acknowledged for inspiration and discovering bugs in the program during the past years, in particular MAIN fan and the second most skilled user Dr. H. Brandstetter. Dr. Lynn Ten Eyck is gratefully acknowledged for providing his FFT routines.

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