

25.2. PROGRAMS IN WIDE USE

Script (among other tools) for visualization of the coordinate data sets.

25.2.8. MAGE, PROBE and kinemages

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25.2.8.1. Introduction to aims and concepts

MAGE and the kinemages it displays (Richardson & Richardson, 1992, 1994) provide molecular graphics, organized in an unusual way, that are of interest to crystallographers for uses that range from interactive illustrations for teaching to a representation of all-atom van der Waals contacts, calculated by *PROBE* (Word, Lovell, LaBean *et al.*, 1999), to help guide model-to-map fitting.

A kinemage ('kinetic image') is an authored interactive 3D illustration that allows open-ended exploration but has viewpoint, explanation and emphasis built in. A kinemage is stored as a human-readable flat ASCII text file that embodies the data structure and 3D plotting information chosen by its author or user. *MAGE* is a pure graphics display program designed to show and edit kinemages, while *PREKIN* constructs molecular kinemages from PDB (Protein Data Bank; Research Collaboratory for Structural Bioinformatics, 2000) files. The latest versions (currently 5.7) of *MAGE* and *PREKIN* are available free for Macintosh, PC, Linux, or UNIX from the kinemage web site (Richardson Laboratory, 2000). The programs operate very nearly equivalently on different platforms and, by policy, later versions of *MAGE* can display all older kinemages. A Java 'Magelet' can show small kinemages directly in suitable web browsers, with their first-level interactive capabilities of rotation, identification, measurement, views and animation.

MAGE has no internal knowledge of molecular structure. A collaboration between the author and the authoring program (*e.g.* *PREKIN*) builds data organization into the kinemage itself. This two-layer approach has great advantages in flexibility, since an author can show things the programmer never imagined, including non-molecular 3D relationships. Overall, kinemages demand less work and less expertise from the reader or viewer than do traditional graphics programs, but that ease of use depends on the effort involved in thoughtful authoring choices, aided by the extensive on-screen editing capabilities described below.

MAGE has been designed to optimize visual comprehension: the understanding and communication of specific 3D relationships inside complex molecules. Display speed has been given priority, to ensure good depth perception from smooth real-time rotation. The interface is extremely simple and transparent, and the colour palette is tuned for comparisons, contrasts and depth cueing. Immediate identification and measurement are always active; views, animations, or bond rotations can be built in by the kinemage author. Text and caption windows explain the intentions of the author, while a simple hypertext capability allows the reader to jump to the specific view and display objects being described; however, most kinemages can also be successfully understood just by exploring what is available within the graphics window.

Kinemages are suitable for structure browsing or producing static 2D presentation graphics, but those aspects have been kept secondary to effectiveness for interactive visualization and flexibility of author specification. Features and representations have deliberately been chosen to be fast, simple and informative rather than either showy or traditional, as illustrated by the following examples and their rationales. Mouse-controlled rotation in *MAGE* depends only on the direction of drag, so that the behaviour of the image is independent of absolute cursor position within the window. Labels are available but seldom needed, since the data structure builds in a 'pointID' that is displayed whenever the point is picked. Instead of using half-bond colouring which

tends to chop up the image, *PREKIN* provides separate colours and button controls for main chain *versus* side chains, and it can prepare a partial 'ball-and-stick' representation with colour-coded balls on non-carbon, non-hydrogen atoms (see Fig. 25.2.8.1). Hydrogen atoms are crucial for some research uses, but to minimize the clutter from twice as many atoms, *PREKIN* sets up their display under button control; in addition, a 'lens' parameter can be specified for the list, allowing display only within a radius of the last picked centre point. For effective perception of conformational change, while avoiding either the confusion of overlays or the potential misrepresentation of computed interpolation, *MAGE* features simple animation switching between known conformations. Very importantly, since molecular information resides mostly in chemical bonds and spatial proximity, kinemages emphasize fully 3D representations, such as vectors, dots, or 'ball and stick's, rather than surface graphics that obscure internal structure. A space-filling representation (the 'spherelist') is available, but it is suggested that it is used very sparingly – for example, to show the size and shape of a small-molecule ligand. If an extensive surface is needed, a dot surface is more informative, since the underlying atoms and bonds can be seen at the same time. Nothing matches a well rendered ribbon for conveying overall 'fold'; *PREKIN* calculates and *MAGE* displays simple ribbon schematics (see Fig. 25.2.8.2) which can be rendered by *Raster3D* (Merritt, 2000) or *POV-Ray* (POV-Ray Team, 2000) for a static 2D illustration, but for interactive use they serve mainly as introduction and context for more detailed 'ball-and-stick', vector and dot representations.

For kinemages, the representation style is not a global choice that applies to everything shown, but rather is a set of local options (varied across space or sequence) chosen to provide appropriate emphasis and comprehensible detail within context.

25.2.8.2. Use as a reader of existing kinemages

Viewing a pre-existing kinemage file requires almost no learning process: the interface is sufficiently 'transparent' that interaction is mainly with the molecule rather than with the program. Six simple operations cover all basic functionalities: (1) drag with the mouse to rotate the displayed object; (2) click on a point to identify it; (3) turn things on or off, or animate if that option is present, with labelled buttons; (4) choose preset views from the Views pull-down menu; (5) read the author's explanations in the text and caption windows; (6) change to the next kinemage in the file with the Kinemage pull-down menu. At a slightly more complex level, one can recentre, zoom the scale, move the clipping planes and save a view; measure distances, angles and dihedrals or 'Find' by point name (from the Tools pull-down menu); change Display menu options such as stereo or perspective; or consult the Help menu. There are keyboard shortcuts for convenience (such as 'a' to animate or 'c' for cross-eye *versus* wall-eye stereo), but they are never the only method and they are defined on the menus. Demo5_4a.kin (Richardson Laboratory, 2000) provides a brief guided introduction to using kinemages.

25.2.8.3. Use for teaching

Simplicity of interface, attention to presentation issues and free cross-platform availability make *MAGE* and kinemages especially well suited for teaching and learning about macromolecular structure or about crystallographic concepts such as handedness and symmetry. Suggestions can be found in Richardson & Richardson (1992, 1994) and in file KinTeach.txt (Richardson Laboratory, 2000). A large body of teaching material is available in kinemage form, including supplements for textbooks on protein structure and general biochemistry, the Protein Tourist files and kinemages for specific papers in *Protein Science*, and a great many web sites involving kinemages, some of which contain course

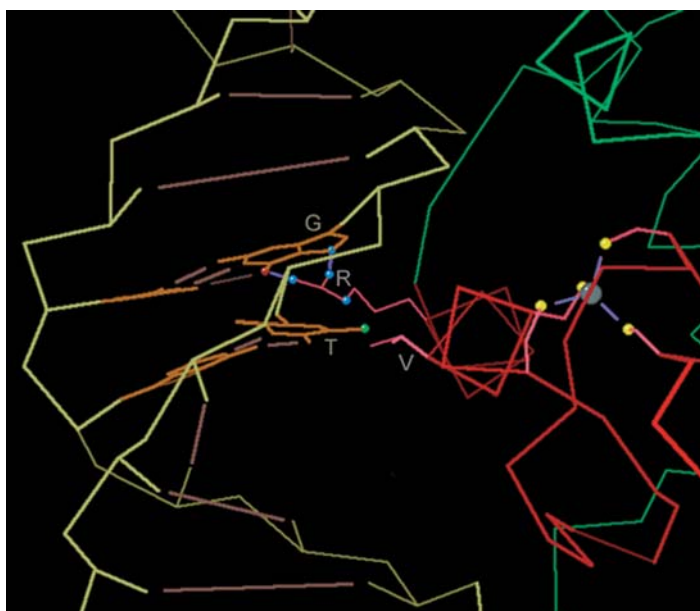


Fig. 25.2.8.1. A typical macromolecular kinemage, combining details with context in the interactive display, for a glucocorticoid receptor–DNA complex (PDB file 1GLU). This view looks down the recognition helix, with one of the 4-Cys Zn sites on the right. Two sequence-specific binding interactions are shown with partial ‘ball-and-stick’ representation: the Arg–guanine double hydrogen bond and the hydrophobic packing of Val to thymine methyl. DNA bases are in gold and protein side chains in pink, while atom balls are colour-coded as N blue, O red, C green, S yellow and Zn grey. Context is provided by the C α backbone for the protein and a virtual backbone for the DNA (using P, C4' and C1'), with lines symbolizing the rest of the base pairs.

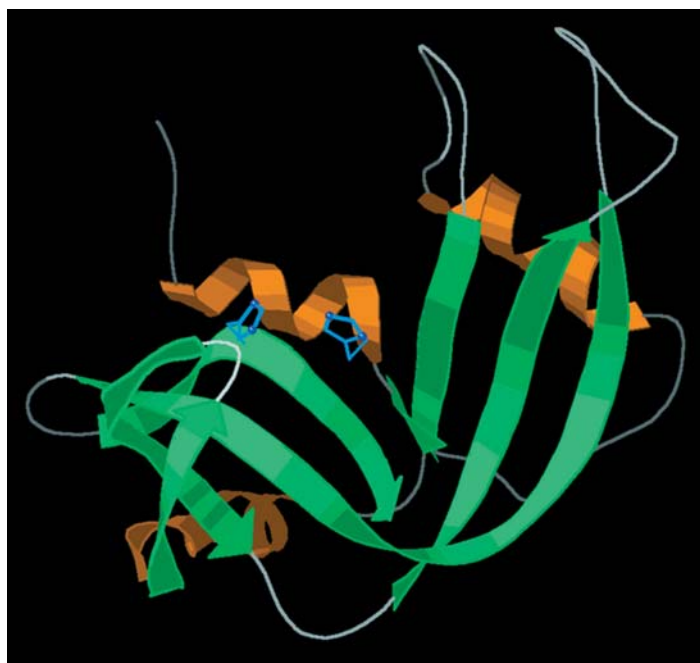


Fig. 25.2.8.2. A ribbon-schematic kinemage of ribonuclease A (PDB file 7RSA), with β strands as sea-green arrows, helices as gold spirals and loops as single splines in white (produced from a built-in script in *PREKIN* and rotatable in *MAGE*). Ribbons have edges to give them some thickness and are shaded rather than depth-cued; C α positions for the active-site His side chains were moved slightly to lie in the ribbon plane.

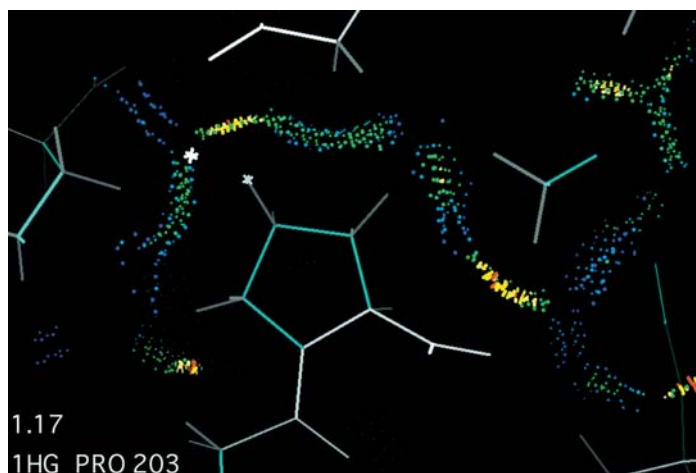


Fig. 25.2.8.3. A thin slice through an all-atom contact kinemage showing the van der Waals interactions of Pro203 and neighbouring atoms in Zn elastase at 1.5 Å resolution (PDB file 1EZM). Contact dots are colour-coded by the gap between atoms: blue for wider gaps (up to 0.5 Å), green for closer fit and yellow for slight overlap (but still favourable). Note the extensive contact and the interdigitation of hydrogen atoms. White markers show the last two points picked (Pro Hg1 and one of its contact dots), while the distance between them and the identity of the last one are shown at bottom left. Hydrogen atoms are from *REDUCE*, contact dots are from *PROBE* and the display is done in *MAGE*.

materials (e.g. Bateman, 2000). References, links and examples can be found on the kinemage web site (Richardson Laboratory, 2000).

25.2.8.4. Use for research

For general molecular-structure studies, kinemages act as a 3D laboratory notebook where author and reader are the same person. These kinemages keep a visual record of the research process with selections, views, labels, measurements, superpositions *etc.*, plus a descriptive record in the text and caption windows. Setting up an animation between conformations or between related structures is an easy and very sensitive way of seeing changes, including correlated motions. Completely new display objects and organizations can be added to kinemages, such as 3D plots of related non-molecular data. Kinemages are an easy and platform-independent way of sharing ideas with collaborators, either side-by-side or at a distance with simultaneous discussion, or just by sending a kinemage with its preset views and notes. Later, the working research kinemages can be used to produce either static 2D or interactive illustrations for lectures or publication.

In addition, *MAGE* and *PROBE* incorporate research tools not yet available in other display systems. *PROBE* analyses molecular interactions by calculating small-probe contact dots wherever two atoms are within 0.5 Å of van der Waals contact (Word, Lovell, LaBean *et al.*, 1999), for numerical scoring or for display in *MAGE* (see Fig. 25.2.8.3), where the three types of contacts (hydrogen bonds, favourable van der Waals contacts and unfavourable ‘clash’ overlaps) are under separate control. Contact-dot analysis requires all hydrogen atoms; they are added by *REDUCE* (Word, Lovell, Richardson & Richardson, 1999), which optimizes the positions of OH, SH, NH₃ and Met CH₃ hydrogen atoms and possible 180° flips of Asn, Gln, or His, considering both van der Waals clashes and hydrogen bonds analysed combinatorially in local networks. These contact-surface tools have research uses that fall into two distinct categories: one is study of the patterns and causes of particular structural features in molecules (best done on atomic resolution structures); the other is sensitive testing, validating and adjusting of an individual molecular model, either computational or experimental. As an example of the latter type, *MAGE* can call *PROBE*