screen. Another graphic program GRAIP (GRaphics of Atomic Information Picture) used to draw a pixel image of the space filling model with its cross section is linked to Mild, using colours to indicate information.
Conformational energy calculation: FEDER (Fast ECEPP and its DERivatives) is a program used to calculate the conformational energy of proteins with respect to dihedral angles based upon the ECEPP function. It finds correct energy minima by a rapid calculation of the first and the second derivatives of the energy function using a new algorithm[1]. Its modified version for an array processor (S-810, Hitachi, 630MFLOPS) is much more effective.
Standard format to describe information of proteins: FLEXS (FLexible, EXtensible and Structural) format is a new format, which can describe any information of proteins with any type and any length, structurally. The Protein Data Bank (PDB) format is widely used, but there are many limitations, especially in describing protein structures by dihedral angles only. The FLEXS format links many program tools, such as between Mild and FEDER. Even when a relational database is available, such a format is still necessary for group work. Examples of the performance of these tools are shown by photos and a film, in which a Monte Carlo simulation of thermal fluctuation[2] of the ovomucoid and the manipulation of Mild are introduced.

References

1 **Abe, H et al.** *Comput. & Chem.* Vol 8 (1984) p 239
2 **Noguchi, Y and Go, N** *Biopolymers* Vol 24 (1985) p 537

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Molecular dynamics simulations: techniques, experimental basis and determination of free energies

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The basic procedures and techniques of the computer simulation method of molecular dynamics (MD) will shortly be reviewed. Its predictive power will critically depend on the basic approximations that are made and the force fields that are applied. Therefore, results of MD simulations of various molecules will be compared to experimental data derived from X-ray, neutron diffraction of 2D NMR experiments. Second, the derivation of the thermodynamic properties, entropy and free energy for systems of interacting particles from simulations will be discussed. This is by no means a trivial task, but recently progress has been made. Examples of the calculation of the free energy of hydration of methanol will be given. Possible application of these techniques to calculate binding constants of inhibitors or substrates to enzymes will be evaluated. As an example the calculation of the relative binding constant of two different inhibitors of dihydrofolate reductase (DHFR) will be discussed.

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Crystallographic approaches to nucleic acid structure and dynamics

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Our knowledge of the tertiary structure of nucleic acids is derived from X-ray diffraction studies of crystalline fibres and single crystals. However, the fact that X-ray crystallography yields well-defined structures characterized by a set of coordinates fosters a rather rigid and static view of nucleic acids. Only recently was it shown that X-ray diffraction can contribute to our knowledge of the dynamic properties of macromolecules. This advance was made possible by the development of refinement methods, which allow the precise determination of atomic coordinates and of the atomic Debye–Waller factors, together with the impetus given by molecular dynamics simulations of macromolecules. Further, the availability of X-ray structures of DNA oligomers and t-RNA molecules at various resolutions has opened up a new era in the study of the structure and biological functions of nucleic acids and of their interactions with other ligands and proteins. The correct interpretation of crystallographic data, especially of low to medium resolution, depends on a thorough understanding of the effects of the refinement model upon the derived quantities, such as coordinates, thermal parameters, and positions of solvent molecules. Several examples of nucleic acid structures refined with molecular graphics, molecular mechanics, or molecular dynamics will be presented and discussed: at high resolution, a Z-DNA hexamer and its solvation; at medium resolution, a comparison between two refinement methods of a B-DNA dodecamer as well as a comparison between two tRNA molecules; at low resolution, a study of heavy metal and drug binding to Z-DNA oligomers.

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MaxTwist: energy minimization of macromolecules in intelligent degrees of freedom

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'Babysitting' irrelevant degrees of freedom is an unnecessary and very time-consuming activity of energy minimization programs. For example, bond stretching requires an energy several times kT, at room temperature, for a single quantum excitation. So there is usually no biological interest in variations in bond length. Yet the most widely used intramolecular minimizers vary all $3*N$ atomic position coordinates, i.e. more than a thousand degrees of freedom for a 58-residue protein. A lot of time is spent changing variables that physically do not change. MaxTwist differs in two respects. 1) The basic description of molecular conformation is in terms of internal coordinates, not atomic position coordinates.

Rigid body constraints can thus be satisfied exactly, rather than by strong restraining forces. 2) Minimization is carried out in any subset or linear combination of these variables. The user's job is to choose intelligently the minimal combination of degrees of freedom appropriate for the problem at hand. Only the chosen degrees of freedom are varied, while all others remain unchanged. The practical advantages of this approach can be considerable. For example, an entire domain of a globular protein can be treated efficiently as a rigid unit, with all surface sidechains freely mobile and interdomain hinges completely flexible. Full flexible geometry is available when needed, e.g. to regularize unreasonable stereochemistry or to extricate the molecule from local minima traps. Workers with experience of protein folding will recognize shades of classical torsion minimizers. In fact, MaxTwist is derived from F32FKMNM incorporating a tree data structure of molecular conformation in terms of bond lengths, bond angles and dihedral angles written in 1972. Energy-minimized and initial structures are compared using the fast superposition algorithm U3BEST. Conformational changes are dramatically displayed in a movie-like manner alternating between different 3D views. This is done with a new molecular graphics program, MacCello, on the vector calligraphics display Wirrwarr. (See abstract 21.)

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Determination of 3D molecular structures from NMR/NOE distance data: application to nucleic acids

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One of the long time goals of NMR spectroscopy has been to determine the 3D structure of macromolecules in solution. Crystals are sometimes very hard to obtain, and in cases where a high resolution X-ray structure is available it is still desirable to know the native solution structure, which may not be the same as in the crystal. The technique which can give the most direct and detailed geometric information about macromolecules in solution is NMR, especially the Nuclear Overhauser Effect (NOE). NOE is a measure of how much a resonance peak belonging to a given nucleus is affected by strong irradiation at the resonance of another nucleus. The main interaction mediating the effect is dipole–dipole coupling, which gives a very strong dependence on the distance between the two nuclei, $N = C\, r^{-6}_{ij}$, so in practice it can only be observed for nuclei that are closer than $\approx$5Å. To reconstruct a structure from a set of distances there are several possible strategies, with the triangulation methods based on a distance geometry algorithm being the conceptually most straightforward. We have chosen to use a molecular dynamics program, complemented with a restraining energy term $E_R(r_{ij}) = K_{ij} \cdot (r_{ij} - r^0_{ij})^2$ which serves the purpose of forcing the atoms i and j close to their observed distance r^0_{ij} during the course of the simulation; at the same time all the other energy terms, describing the covalent structure and van der Waals characteristics, keep the system in a stereochemically reasonable conformation. Molecular dynamics (MD) in this application has the advantage over energy minimizations that a larger fraction of conformational space is searched, which makes MD less sensitive to the choice of starting conformation. The method has been applied to the determination of the 3D structure of a short piece of DNA, $d(CGTACG)_2$, for which 190 interproton distances had been estimated in NOE experiments. It is possible to show that irrespective of whether the simulations were started from a classical A-DNA or a B-DNA, the resulting conformations after a few picoseconds were essentially the same in the two cases: a structure of the B type, but with more local structure variations than in the ideal structure from fibre data.

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Simulation and 3D display of DNA–histone complexes

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Recent developments in equipment and software make possible the interactive display of more than 10 000 atoms so that specific features of large macromolecules may be simulated to investigate long-range spatial relations, correlations of binding sites and dynamic effects. The first step towards these goals is the creation of structures that simulate macromolecules while preserving their most essential structural features, nevertheless allowing, whenever possible, the use of simple mathematical procedures for their construction. An acceptable balance between the need for structural fidelity and the requirement for simplicity in construction must be aimed at. This problem is particularly acute for all those structures which are known to occur in several slightly different conformations or in minor variants of chemical composition. The work to be presented shows simulations of DNA and histones which serve as the basis for structural refinements and investigations of their interactions. A program has been developed which constructs nucleic acid superhelices of variable pitch and radius. This program does not simply bend a straight piece of nucleic acid by applying a geometric transformation to all atoms[1,2], but rather it projects individual basepairs without distortions to their positions in the superhelix. Therefore, the coordinates of the nucleotides are preserved in their standard configuration[3] and it is only necessary to refine the backbone linkage. The program allows the computer controlled simulation of any type of DNA sequence bent along any desirable radius without distortion of internal bonds or angles. It thus becomes possible to study the effect of smooth bending of DNA on the width of its grooves,