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Special Topic

Molecular Modeling Software and Methods for Medicinal Chemistry†

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I. Introduction

Molecular modeling has become a well-established research area during the last decade due to advances in computer hardware and software that have brought high-performance computing and graphics within the reach of most academic and industrial laboratories. A growing number of journals now focus on molecular modeling: *Journal of Computational Chemistry, Computers in Chemistry, Journal of Computer-Aided Molecular Design, Journal of Molecular Graphics, Molecular Simulations,* and *Tetrahedron Computer Methodology.* Several recent texts and reviews describe progress in molecular modeling research and applications.[1-7]

This review is intended to provide medicinal chemists with introductory material related to available molecular modeling software and methods. A particular emphasis is given to current software that integrates multiple methods, including graphic and computational tools, and focuses on systems familiar to the committee.

It is important to realize what is really meant by "computer-assisted drug design". Molecular modeling systems provide powerful tools for *building, visualizing, analyzing,* and *storing* models of complex molecular systems that can help interpret structure–activity relationships. The critical problem of *molecular design*—what structure do we build, model, and possibly synthesize?—is not answered by current methods and is left up to the creativity of the medicinal chemist. The goal of molecular modeling should not be limited only to providing insight, but it should also help to suggest new experiments, i.e., new structures tailored to have the desired biological activity. Molecular modeling cannot yet produce quantitative predictions of activity except in very special cases, but it can provide valuable qualitative guidelines that help design new lead structures. The result of a successful modeling study is therefore usually one or more candidate structures predicted to fulfill particular criteria described in a molecular model, i.e., a pharmacophore. The synthesis and biological evaluation of these target structures can be used to test and iteratively refine the model.

"Direct" and "indirect" design are the two major modeling strategies currently used in the conception of new drugs. In the first approach the three-dimensional features of a known receptor site are directly considered, and in the latter the design is based on the comparative analysis of the structural features of known active and inactive molecules that are interpreted in terms of complementarity with a hypothetical receptor site model (Figure 1). Spe-

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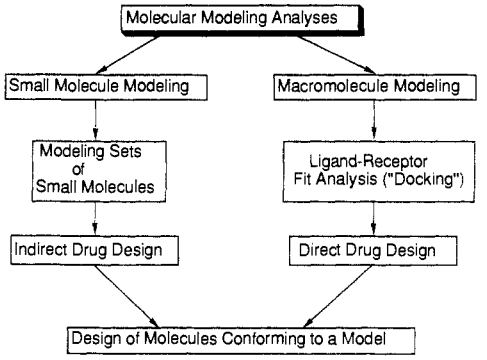

Figure 1.

cialized molecular modeling systems have been developed to analyze either the interaction of a prototype molecule with a known receptor site or the ability of a given compound to mimic the three-dimensional stereochemical features of known active compounds. Both approaches attempt to optimize receptor fit for selectivity and binding affinity while qualitatively considering other critical factors (log P, solubility, metabolic stability, etc.)

Most molecular modeling systems strive to provide the same basic set of features: visualization and manipulation of three-dimensional molecular models including rotatable bonds, structure building, molecular mechanics and/or dynamics, conformational analysis, electronic properties, molecular surface displays, and the calculation of various physical properties.

II. Interactive Graphics Display and Manipulation

A large range of graphics workstations are available to meet the needs of modeling applications ranging from simple, small molecule to complex macromolecules. For small molecules basic, inexpensive systems may be adequate (e.g. a Macintosh II can handle up to a couple hundred atoms in real time; real time means that the molecular model rotates and translates smoothly under interactive control). Current personal computer (PC) molecular modeling software have been reviewed recently.[175,187] The sheer size of macromolecules requires sophisticated graphics software and hardware to provide real-time, interactive response along with selective display and manipulation.[8] Current state-of-the-art systems are capable of simultaneously handling up to 20 or more molecules with up to about 20 000 atoms and thousands of molecular surface points in real time with depth-cued color and time-sliced stereo. Each molecule should be able to be individually labeled, color-coded, and controlled in three dimensions, while simultaneously monitoring inter and/or intramolecular distances and adjusting multiple contiguous or noncontinguous dihedral angles. Dials, joysticks, and a mouse, or an excellent new interactive device called "Spaceball",[9] which simultaneously control all six degrees of rotational and translational freedom with a single hand, are used to translate and rotate molecules and to rotate bonds. Typical operations are activated by first pointing to a menu and next to atoms and bonds, either with a stylus or a "mouse" to calculate, for example, distances and angles (dihedral or valence). Most systems continually update this information as the geometries are modified. The latest graphics workstations have very fast processors that do complete bump-checking (checking for contacts closer than van der Waals) and even molecular mechanics and dynamics energy calculations in real time (for small molecules up to about the size of a decapeptide). Selective control of which molecules or portions of molecules are displayed and which molecules, distances, and dihedral angles are active requires a powerful command language along with interactive "picking" of atoms and bonds with a mouse or stylus.

The trend in recent molecular modeling software design has been to exploit the powerful new windowing and computational power of the new generation of graphics workstations. This has resulted in an emphasis on menu-driven systems, which are intuitive and easy to learn, but sacrifice generality and completeness if not carefully implemented. Menu designs provide the most basic commands, but the complex syntax required by nearly all the current systems' command languages makes specifying functions not found on the menus cumbersome, if not impossible, for the nonspecialist. Hopefully, continued software design efforts will create improved menu systems and realize the need for simple, English-like command language syntax to supplement features not easily implemented in menus. The new design trend has also focused on integrating computational chemistry (e.g. molecular mechanics and dynamics) with graphics display, but much of the effort has been devoted to computations, at the expense of neglecting important features and a good user interface for interactive graphics pioneered in previous generations of graphics-only modeling systems. Despite the impressive computational performance of the new workstations, even the most sophisticated techniques provide only rough, qualitative guidance for most medicinal chemistry applications. Good interactive graphics with a well-designed user interface maximizes the performance of the most critical part of the system—the chemist.

Raster graphics has recently become the dominant technology in interactive molecular modeling, replacing the older calligraphic or vector display systems. Although raster displays have apparent advantage in providing beautiful "realistic" color solid shaded images, these images cannot be updated fast enough (with transparency and clipping) for real-time modeling yet, so vector and dot images (on raster displays) still provide the best approach for high-performance molecular modeling. Vector (bonds) and dot (molecular surface) images have the tremendous advantage of providing full transparency and clipping while displaying a complex, color-coded molecular surface and bonds, which are essential for studying interactions deep inside a macromolecular binding site or comparing several small molecules.[8] Time-sliced stereo, where the left and right eye views are alternately displayed approximately every $^1/_{30}$ s and viewed through a mechanical shutter or liquid crystal glasses synchronized to the display, provides a very convincing three-dimensional illusion and is extremely helpful for modeling complex interactions. A recent major improvement in stereo viewing is to place a liquid crystal screen over the entire graphics screen, allowing the user(s) to wear circularly polarized plastic glasses.

The simultaneous development of real-time interactive color graphics[8] and Connolly's molecular surface program[10] in 1980 revolutionized molecular modeling. Color-coded surfaces provide qualitative displays of hydrophobic and hydrophilic regions, neutral and charged groups, electro-

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static potential, and mobility (based on X-ray crystallographic refinement or molecular dynamics simulation). Color-coded molecular surfaces therefore simultaneously display the main features critical to receptor binding: shape, charge, and hydrophobicity. Hydrophobic color coding was originally done simply by coloring all surface points associated with carbon "hydrophobic" (e.g. red) and all nitrogen and oxygen surface points "hydrophilic" (e.g. blue); an improved approach[11] includes "neutral" surface (e.g. yellow) for sulfur, α-carbons of amino acids, the carbon between the imidazole nitrogens in histidine, and carbonyl carbon. Molecular surfaces can also be color coded by a so-called "hydrophobic potential", based on fragment hydrophobicity values and a simple empirical function analogous to the classical formula for electrostatic potential.[12,13] Electrostatic potential molecular surfaces[14] are calculated using quantum mechanically derived partial atomic charges for each atom.[15] The potential is usually calculated one probe sphere radius above the molecular surface to give a qualitative view of what an incoming ligand "sees" as it approaches the macromolecule. The surface is color coded by the value of the electrostatic potential at each point. The electrostatic potential gradient or electric field can also be displayed graphically using short vectors.[16] Similar representations can also be envisaged for any other potential or field such as, for example, the molecular mechanics potential experienced by different chemical probes.[139]

Connolly's program[10] implemented Richard's definition[17] of molecular surface by rolling a probe sphere (usually 1.4-Å radius, the effective radius of water molecule) over the surface of the molecule, resulting in a smooth surface that represents the surface accessible to a water molecule, including internal cavities. Langridge's UCSF group[18] and Pearle and Honneger[19] independently developed van der Waals dot surface programs that are much faster than Connolly's molecular surface program, although they are not as effective at eliminating buried surface and produce a more complicated surface display for macromolecules. Both types of surface are available in most modeling systems. Connolly also developed an analytical method for calculating molecular surface,[20] which provides nearly exact values for the surface area and volume[21] enclosed by a surface along with spectacular shaded raster graphics images,[22] which gives a much different impression of a surface than the conventional CPK-like raster surfaces.[23] Barry introduced the very useful "extra radius" surface,[24] where the surface is calculated one van der Waals radius beyond the normal surface, collapsing the surface of a binding site onto the vector model of its ligand and eliminating the need for displaying the ligand's surface. This simple graphics trick makes it much easier to visualize the "docking" of a ligand into a binding site. For example, chymotrypsin's specificity for aromatic amino acid side chains is not immediately apparent from a conventional molecular surface of its active site, while the "extra radius" surface reveals an almost perfectly planar rocket that is obviously complementary to an aromatic ring. The "extra radius" surface can also be color coded by hydrophobicity or electrostatic potential.

III. Small Molecule Modeling

(a) Structure Building. Every system should provide means allowing one to construct accurate three-dimensional models of organic molecules. One of the simplest and most reliable ways is to use libraries of typical organic fragments and the Cambridge X-ray Crystallographic Data Base,[25] which contains about 50 000 structures. A molecule is constructed by assembling preexisting fragments, followed by successive adjustments of the current structure, which allows the user full control over building a reasonable starting conformation with the desired stereochemistry. Several common building functions were involved in these operations: make-bond, break-bond, fuse-rings, delete-atom, add-atom, add-hydrogens, invert chiral center, etc. They are combined with continuous refinements of the geometry of the current structure using molecular mechanics.

Most systems have facilities allowing one to draw chemical structures as a two-dimensional sketch describing the atom types (element and hybridization) and connectivity (what's bonded to what), along with some method of specifying stereochemistry (up/down, R/S, etc.). While in principle a simple and intuitive approach, it has proven very challenging to design robust methods to convert the initial two-dimensional information into reasonable low energy conformations. Most of these approaches are molecular mechanics, but often become trapped quickly in poor local minima during the conversion from two into three dimensions. Distance geometry combined with molecular mechanics[26,27] usually provides superior results to molecular mechanics alone. Very few systems are able to handle the conformational multiplicity of cyclic moieties in a fully automatic manner.[72,159] Pearlman[28] recently introduced CONCORD, an elegant method for rapidly generating good quality three-dimensional structures directly from a SMILES[29] code (a simple alphanumeric language for encoding organic structures). CONCORD is currently the best available method for generating small-molecule three-dimensional structures interactively, due to its ease of use, speed, and the quality of the resulting structure. It has the advantage of being able to produce a good quality structure for most organic compounds, including those with complex heteroatom functional groups and ring systems, without the need for developing molecular mechanics parameters. However, CONCORD

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generates only a single conformer and cannot be used for conformational sampling. CONCORD has also been used to generate three-dimensional structures from two-dimensional structures stored in large industrial databases to provide conformations for newly developing three-dimensional search techniques.[30]

Many popular file formats for storing three-dimensional coordinates are in use (Brookhaven Protein Data Bank, Cambridge, Molecular Design's MOLFILE, CHEM-X' CSSR, etc.), but unfortunately there is no accepted convention or standard. The best current solution, used by more and more modeling systems to provide compatibility with other software, is to include facilities to read and write most or all of the popular formats, while making it easy for the user to add new formats. A standard molecule file format has been proposed.[160]

Molecular modeling studies result in a proliferation of files containing different results from different theoretical and experimental methods. Keeping track of all this data for several different projects can easily become a bookkeeping nightmare. Several current systems provide simple databases for storing and retrieving the results generated. A more general solution is provided by THOR,[32] an elegant chemical database system based on SMILES[29] codes. Martin et al.[33] described the use of THOR for molecular modeling.

(b) Molecular Mechanics. Molecular mechanics methods[34,35] are based on a pragmatic view of the molecular structure that is considered as a set of balls and springs with series of potential energy functions expressing the molecular force field as a sum of these functions. A typical energy equation is as follows:

$$E_{\text{total}} = E_{\text{stretching}} + E_{\text{bending}} + E_{\text{dihedral}} + E_{\text{van der Waals}} + E_{\text{electrostatic}} + E_{\text{hydrogen bond}}$$

Each of the individual energy terms have preferential equilibrium positions (bond lengths, bond angles, dihedral angles, van der Waals interaction distances, etc.) and force constants that are either experimentally known or theoretically estimated and used to associate energetic penalties with each individual deviation. A "Force Field" therefore consists of a set of analytical energy functions and their associated sets of numerical parameters. The total energy of a given molecule can be the sum of several thousands of individual contributions. Force field development remains a major problem for the large variety of complex functional groups encountered in medicinal chemistry, which is further complicated by the fact that not all force fields are readily transferable from one package to another. The most extensively tested force fields are MM2[34] (hydrocarbons plus a limited selection of simple heteroatom functional groups), AMBER[36-38] and CHARMM[39] (peptides and nucleic acids), and ECEPP[173,174] (peptides). MM2 is the current standard for small-molecule work, but is a poor choice for macromolecules. AMBER and CHARMM force fields are similar and are the standard for macromolecules, but give only qualitative results on small molecules. Hybrid force fields, such as the AMBER all-atom force field,[38] are usually used for calculations involving small-molecule–macromolecule interactions. Molecules that contain functional groups not parameterized by the above force fields require the estimation of new parameters specific for each new bond, bond angle, or dihedral angle type.[40] Most of the major software systems provide facilities for automatically assigning the appropriate atom types and parameters, but there is considerable variation in the quality and quantity of the parameters available. It is always prudent to calibrate unfamiliar software with some well-known test cases. Biosym[41] has formed an industrial consortium to systematically develop and test force field parameters. Assuming that all the necessary parameters are available for a given molecule, relative total strain energies can be calculated for estimating rotation or inversion barriers, preferred conformations, the energy required to achieve a specific conformation, etc. Except for special cases (e.g. estimating the enthalpy of formation of a hydrocarbon) the absolute calculated energy is of little value—*relative* energies between different conformers or isomers are important. The texts by Buckert and Allinger[34] and Clark[42] provide an excellent description of molecular mechanics and its applications.

Molecular mechanics energy minimization involves successive iterative computations, where an initial conformation is submitted to full geometry optimization. All parameters defining the geometry of the system are modified by small increments until the overall structural energy reaches a local minimum. The goal is to reach a local minimum on the potential surface within the minimum amount of time. The more sophisticated methods use the first and occasionally the second derivatives of the energy function for guiding the minimization. No method can guarantee finding the absolute lowest energy structure—the global minimum. Energy minimization will stop at the first local minimization encountered, without realizing that much deeper, more stable minima may be accessible. The problem is analogous to a ball rolling downhill, which stops in the first valley it finds and is unable to climb the next hill which may lead to a deeper valley. Molecular dynamics is able to climb small barriers (the barrier height depends on the temperature of the dynamics simulation) and is therefore much more efficient at locating deep local minima than simple minimization; short dynamics runs are now commonly used for minimization. Systematic search,[43,44] which increments all rotatable bonds in turn to explore the complete conformation space of the molecule, distance geometry[45,46] and

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other random sampling approaches attempt to locate the global minimum through thorough exploration of the allowed conformations, while the ellipsoid method[47,48] and an extension of distance geometry called energy embedding[49] can accomplish near global optimization in some cases.

Energy minimization can proceed either in internal coordinates (the variables explicitly considered are the bond lengths, bond angles, and dihedral angles) or, as is more often the case, in Cartesian coordinates (each atom is characterized with x, y, and z coordinates, and the atom moves with small increments along these axes). An advantage of minimizing in internal coordinates is that cooperative movements of several atoms or groups are well simulated in such treatments; moreover since the degrees of freedom of the chemical structures are natural, the risk that the molecules are trapped in a false minima is greatly reduced.

(c) Molecular Dynamics. In the last 10 years the static views of molecules have been considerably enlarged to include new perspectives introduced by molecular dynamics.[50,51] X-ray crystal structures represent a time-averaged structure of a continuously moving system, while molecular dynamics simulates the actual, instantaneous motion of the system. Each atom is treated as a particle responding to Newton's equations of motion: successive integrations of these equations lead to the trajectory of the atom over time in the form of a list of positions and velocities. Analyses are made through periods of typically 1–100 ps (many interesting motions are fully developed within 100 ps or less).

The motions of the atoms and chemical groups obtained by these simulations reveal subtle underlying molecular machinery and make it possible to understand phenomena that cannot be explained by the static view. Over short periods of time (e.g. a fraction of a picosecond), molecular dynamics usually shows little coherence in the displacements of the atoms. The motions are frequently interrupted by collisions with neighboring groups, and each group seems to have an erratic trajectory. Over longer periods of time, coherent and collective motions start to develop, revealing how some groups can fluctuate somewhat more than others.

The calculations require good computational power as well as appropriate graphical facilities. Animation consists of the viewing of consecutive conformations generated by molecular dynamics calculations. Animated display of molecular dynamics simulations is essential; dynamics simulations produce huge amounts of data that are difficult to interpret without graphics.

Moelcular dynamics is useful in order to identify preferred motions of either small molecules or proteins. Although it is not of direct utility in drug design except for "where does it spend most of its time" and as an improved energy minimization approach, dynamics gives a high information content picture of the precise behavior of the molecule considered and the way it can behave and interact with other partners. Restrained molecular dynamics[52] adds an artificial penalty function to restrain specific distances, angles, or dihedral angles. Restrained molecular dynamics and distance geometry[53,54] have been used to generate three-dimensional structures of small molecules, proteins, and nucleic acids consistent with NMR data.[55] Multiple energy minimization force fields are used in molecular dynamics methods and have been described in the literature.[178-186] Recent reviews[176,177] provide excellent description of molecular dynamics and related methods and illustrate various application approaches.

(d) Quantum Mechanics. In principle all treatments mentioned in the preceding paragraph can be made by using quantum chemical calculations. Molecular energies are calculated by using the Schroedinger equation with the Molecular Orbital (MO) formalism, which can provide greater accuracy along with the ability to model electronic effects not treated by molecular mechanics, as well as consume enormous amounts of computer time depending on the method and approximations used. Over a long period of time the Quantum Chemical Program Exchange (QCPE) group located at the University of Indiana has contributed greatly to the dissemination of a number of excellent theoretical chemistry programs to the scientific community.

The Schroedinger equation of a given molecular system can be solved either with no approximations at all (ab initio) or with the introduction of some approximations (semiempirical). Semiempirical treatments such as AM1,[56] MNDO,[57] CNDO[58,59] INDO,[60] EHT, MINDO,[61] PRDDO,[62] and PCILO[63,64] are some of the most popular semiempirical programs, whereas the GAUSSIAN[65] and HONDO[66] series are typical ab initio programs. AMPAC and MOPAC are QCPE packages that include the AM1, MNDO, and MINDO programs. Along with GAUSSIAN series, these are among the most popular programs for quantum mechanical calculations.[67]

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Energies can be obtained through either the "self consistent" (SCF) formalism or with "perturbation methods". The SCF method is based on a property of the Schroedinger equation which states that whatever wave function is used to calculate the electronic energy of a given system, the corresponding energy will always be greater than the true energy value. SCF treatments are based on that property as follows: starting with an initial wave function, iteratively modify it until the total energy does not decrease. Full geometry optimizations therefore require the combination of two types of minimization: one for the calculation of the energies, and one for the optimization of the geometries.

In the perturbation methods, as in PCILO approaches,[63,64] the total energy is calculated as a convergent series of terms, with each new term improving the accuracy of the previously computed energy. The approach starts from the initial two-dimensional chemical formula that is used to compute the first term of the series. In general the treatment is stopped either at the second or at the third order. An advantage of these computations is that they are relatively rapid and permit one to obtain "conformational maps" (e.g. energy contours according to the variation of two dihedral angles). The computer time necessary to calculate a map using a 30-deg increment ($12 \times 12 = 144$ conformations) is comparable in perturbation methods to the time necessary for only one or two conformations using SCF methods.

Quantum chemical calculations can provide detailed insight into the electronic nature of the molecular structures and allow one to analyze phenomena not yet parameterized for molecular mechanics. Molecular mechanics calculations compete favorably with MO calculations for conformational analysis and can be applied to much larger molecules; however, there are a number of physical, chemical, and electronic indices that can be obtained only with quantum mechanical treatments. These methods are theoretically powerful and can be very useful, but the tremendous amount and variety of data they generate must be interpreted with care. In some treatments, particularly when it is known that different methods might not lead to the same results, it is safer to pay more attention to the variations and the trends of the molecular property analyzed rather than to consider their absolute values. A well-known example of lack of agreement of different methods is the calculation of partial atomic charges, which are required by most molecular mechanics force fields and for the calculation of molecular electrostatic potentials. Several approaches have been developed for calculating partial atomic charges in molecules.[15,68-70] Current knowlege of the strengths and weaknesses of available semiempirical and ab initio methods was recently reviewed in an excellent introductory text.[42] Richards' text[71] provides a good introduction into applications of quantum mechanical calculations for medicinal chemistry.

In practice only molecules containing less than about 50 atoms can be studied with quantum mechanical approaches. The selection of the most appropriate method depends not only on the size of the molecule but also on the type of molecular property (e.g. conformation, electronic density, electrostatic potential, frontier orbitals, etc.) that is desired. Most major molecular modeling software packages provide interfaces to popular quantum mechanical methods.

(e) Conformational Analysis. In a first approximation, only intramolecular forces are considered to calculate the conformational properties of a given molecule. However, force field treatments are not restricted to isolated molecules ("gas phase simulations"), they can be envisaged with two molecules as in "docking" analyses, or even simulate solvent molecules in the investigation of solvent effects. Since the global energy minimum is not necessarily the receptor-bound conformation, it is essential to sample a region up to several kilocalories/mole above the global minimum. Molecular mechanics approaches are commonly used for conformational analysis, but quantum mechanical methods can be used for small molecules with two to three rotatable bonds.

A multiple conformation generation function appears now in an increasing number of modeling systems, but is often restricted to the rotation of acyclic bonds. Few modeling systems are able to handle the conformational multiplicity of cyclic (monocyclic or polycyclic) systems automatically. A robust method based on conformational assembly rules has been described[72] allowing the systematic and automatic generation of possible conformations of simple or complex cyclic molecules having, for example, precise polycyclic fused, spiro and bridge-headed systems (when the size of the rings is relatively small, e.g. less than eight members for each elementary ring). Smith et al.[73] described a variation of systematic search for cyclic systems. Gerber et al.[158] developed an elegant method for the systematic generation of conformations in macrocyclic systems that is based on generic shapes approximated by Fourier harmonic representations. More general methods based on artificial intelligence techniques were proposed to generate reliable low-energy conformations of any given small molecule.[74] Efficient variations of systematic search techniques have been described by Dammkoehler et al.[43,44] and Lipton.[75] Chang et al.[128] recently described a new Monte Carlo (random) torsion search method that appears to be one of the most efficient approaches for small molecule conformational analysis. Most major molecular modeling systems include approaches, along with extensive analysis facilities (e.g. contour plots of energy as a function of two dihedral angles). Scheraga and Colleagues have developed a series of techniques in conformational searching of polypeptides (for a review, see ref 169) that include build-up procedures,[170] increase of dimensionality,[171] Monte Carlo plus minimizations,[172] and optimization of electrostatics.[169]

Distance geometry calculations can also be used to generate random starting conformations for conformational analysis.[26,27] Distance geometry is a general method for converting a set of distance constraints into a set of three-dimensional coordinates consistent with the constraints.[45,46] The distance constraint matrix describes the complete conformation space of a molecule by including the maximum possible distance (upper bond) between each atom pair and the minimum possible distance (lower bound). All possible conformers lie between these upper and lower distance bound—distance geometry converts this distance information into three-dimensional coordinates.

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Distance geometry produces a random sampling of conformation space by selecting random distances within each pair of upper and lower bounds. This approach samples conformation space rapidly and efficiently, but cannot guarantee that all of conformation space has been searched. Systematic dihedral search methods can in theory promise that all conformation space is adequately searched, but in practice, the completeness of the search is limited by the increment used in the dihedral scan. The time required for systematic search increases exponentially with each additional rotatable bond and becomes impractical beyond 12–13 rotatable bonds. The time required for distance geometry is independent of the number of rotatable bonds and depends only on the total number of atoms; distance geometry has approximately a quadratic time dependence on the number of atoms and therefore is still practical for large structures that are beyond the reach of systematic search methods. Cyclic structures are handled naturally by distance geometry with no decrease in efficiency, but systematic search method must deal with the ring-closure problem which further limits their efficiency and range.[73] Both methods require molecular mechanics calculations to calculate the energy of each generated conformation; systematic search methods often use a single-point energy calculation since bond lengths and angles are not distorted from their ideal values, but distance geometry requires at least partial energy minimization since all degrees of freedom are varied. Distance geometry is currently not available in any major molecular modeling software system, but stand-alone programs are available commercially,[76] from QCPE[53,77] or from UCSF.[78]

The ellipsoid algorithm is a promising new approach for generating low-energy conformations of molecules by efficiently sampling among the sterically allowed combinations of dihedral angles. It has been applied to the conformational analysis of 18-crown-6,[79] the determination of peptide solution structure using NMR distance constraints,[47] and ligand–protein docking.[48] For small to medium-sized molecules it may be more efficient than either systematic search or distance geometry for locating deep energy minima.

(f) Physical Properties. Although conformational analysis constitutes one important aspect of molecular modeling, a number of physical properties are also accessible with theoretical calculations. Molecular mechanics, semiempirical, and ab initio methods[42] can give rather reliable results on various molecular properties such as heats of formation, enthalpies (e.g. in evaluating the relative stability of isomers), barriers and activation energies, dipole moments, reaction paths, etc. Theoretical calculations can provide a number of indices that may not be directly related to experimental data but that can be very useful because they carry high physical information content (molecular, localized, and frontier orbitals, electronegativities, polarization, delocalization, atomic and bond population, etc.). For example, electron densities are useful because they provide a good basis for the analysis of the stereoelectronic properties of either isolated or interacting molecules. Molecular electrostatic potentials are usually generated from the partial atomic charges derived from a quantum mechanical calculation. Most of the major software systems include facilities to calculate and display electrostatic potentials. Other properties can be calculated by empirical methods; the most popular are the prediction of log P (octanol/water partition coefficient) and MR (molar refractivity) as developed by the Pomona College Medicinal Project.[80,81]

IV. Modeling Sets of Small Molecules

In indirect drug design the modeling is based on the recognition of three-dimensional stereochemical features common to sets of active molecules—the pharmacophore. Superposition and comparison methods, often called "molecular fitting" or "pharmacophore alignment", are the most routinely available. They compare, on a pairwise basis, an active reference compound with a set of other structures. Excluded volume analysis[82] is a classical way to geometrically compare a set of active and inactive molecules in order to reveal essential features, based on the simple idea that regions of inactive molecules which protrude beyond the volume common to the active molecules indicate sterically unfavorable regions on the receptor. The most popular approach to phamacophore superimposition has been the "active analogue" approach, developed by Marshall et al.[83,84] which uses systematic search to determine the allowed conformations of all molecules in the study, followed by comparison of interatomic distances to select conformers that overlap, based on the proposed pharmacophore. Attempts to take into consideration the conformational energies during the fitting process have been made.[85,86] The more recent "ensemble distance geometry method[77,87] will rapidly determine if any solutions exist without replacing a complete systematic search and, if so, provide a random sampling of solutions that indicates how uniquely determined the model is. Additional advantages of this approach are that it handles rings naturally without the ring closure difficulties encountered in dihedral search methods and that chirality can be allowed to vary for any stereo centers of unknown absolute configuration.

Most available systems provide simple interactive fitting functionality by considering the molecules as conformationally rigid, while optionally allowing motion of a few dihedral angles.[85,88] Most of the major software systems

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have integrated flexible fit computational modules in which not only the internal rotational degrees of freedom but also the conformational energies of the individual molecules are taken into account. MAXIMIN[85] is an example in which two alternative methods are possible: a set of flexible molecules can be mapped onto a rigid reference compound, or all the molecules are treated as flexible entities, and the treatment is directed toward the minimization of the conformational variance of the whole set. "Template forcing"[89] is another way to maximize overlaps between molecules using restrained molecular mechanics and dynamics.

In molecular fitting treatments the maximization of the overlaps is generally achieved by geometrical least-squares minimizations, which requires a preliminary selection of pairs of atoms expected to be superimposable. The choice of the pairs of atoms is very subjective, on the basis of "chamber intuition" and the hypothesized pharmacophore. Less subjective approaches have also been developed, on the basis of maximizing the overlap of a set of molecules by minimizing the exposed area of the entire set while simultaneously ensuring that the energies of the individual molecules remain close to a local minimum,[86] combinatorial methods for comparing all possible overlaps of similar atom types,[90,91] and approaches based on three-dimensional electrostatic potential similarity,[92,93] molecular surface similarity,[94] and molecular shape analyses.[161-164]

A more physical approach is to force common pharmacophore atoms to interact with a common binding site, defined by hypothetical points of interaction (e.g. dummy atoms), rather than forcing them to directly superimpose. Different chemical moieties can be compared and do not need to be exactly superimposable.[155,156] Several systems provide Boolean logical operators (and, or, not, etc.) which allow one to find common similarities between two molecules in terms of preselected electrostatic contours or molecular volumes. Cramer et al.[95] recently described a promising new 3D-QSAR method based on calculating the interaction of each molecule in a set of superimposed active structures with a variety of probe atoms on a three-dimensional lattice.

New approaches developed on databases of minimized conformers and using three-dimensional substructure and similarity search techniques[30] have already shown value in identifying pharmacophoric moieties and associated active conformations of molecules.[33] Efforts of this type are current topics of modeling development and are just now becoming available.

V. Macromolecule Modeling

X-ray crystallography and macromolecular modeling provide the most detailed possible view of drug–receptor interactions and have created a new, rational approach to drug design where the structure of a drug is designed on the basis of its fit to the three-dimensional structure in the receptor site, rather than by analogy to other active structures or random leads.[96,97] There are now over 300 X-ray crystal structures of proteins and nucleic acids that have been solved; most are available in the Brookhaven Protein Data bank,[98] including several ligand–macromolecule complexes. Although relatively few structures of actual or potential drug receptors have been solved, the rate of solving these structures has increased steadily during the last few years and will continue to increase due to improvements in crystallographic techniques and the availability of new protein through recombinant DNA approaches. Such high-resolution structures offer the potential of designing drugs tailor-made to fit their receptor with high affinity and selectivity. However, the rate of release to the public domain of three-dimensional coordinates of important macromolecules is decreasing even as the rate of solving them increases. The results of the technology that promised this great potential for rational, receptor-based drug design are in fact often not available. The issues surrounding this counterproductive situation have been discussed previously.[99,100]

Despite the impressive advances in macromolecular X-ray crystallography, availability of high-quality crystals remains the major limiting factor. 2D NMR techniques have advanced tremendously[55,101,102] and can now provide three-dimensional structural information on small proteins (up to 100–150 residues) and DNA in solution, using distance geometry[53,54] and/or restrained molecular dynamics[52,103] to build models consistent with distance constraints derived from NOE (nuclear overhauser enhancement) and coupling constant data.[55] In several cases 2D NMR has been used to solve a complete protein structure; Tendamistat, the 75-residue α-amylase inhibitor, was solved independently by 2D NMR[104,105] and X-ray crystallography,[106] resulting in very similar structures. 2D NMR previously provided only low-resolution models that revealed the overall folding pattern with little information about side-chain locations, but Wuthrich's group has recently determined the complete solution structure of Tendamistat by NMR, including all side chains[105]. The January 1989 release of the Brookhaven Protein Data Bank[98] includes for the first time a protein structure solved in solution by NMR; other structures solved by NMR will follow.

Most current software systems provide efficient means for the construction of polymeric fragments. Peptides, nucleic acids, or carbohydrates are easily generated in an arbitrary or user-defined three-dimensional conformation by selecting in a menu the linear sequence combined with additional information indicating how the progressively growing molecule should fold. The growth either can be fully extended or can follow commonly observed secondary structure (e.g. α-helix, β-sheet in the case of peptides; A,

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B, or Z conformation for nucleic acids, and analogous prespecified conformers for carbohydrates). These simple methods have little chance of leading to meaningful three-dimensional structures unless they are used in combination with additional knowledge and experimental data.

Many more protein sequences are available than crystal structures, and the gap will continue to grow as DNA-sequencing methods become even faster. Fortunately, protein sequences occasionally show high sequence homology with proteins whose three-dimensional structure is known, suggesting the possibility of modeling the unknown structure based on the crystal structure of the homologous protein. This has become a popular approach and has recently been reviewed by Blundell et al.;[107,108] an example is the recent prediction of the three-dimensional structure of tissue plasminogen activator.[109] Homology modelling techniques have been particularly successful for predicting antibody structures.[110,111] Jones and Thirup[112] showed that it may be possible to fit most secondary structure elements using fragments from other proteins of known structure; this approach is useful for building models for insertion and deletion regions and for homology model building in general. Most of the macromolecular modeling software systems contain similar facilities for protein homology modeling.

For the majority of protein sequences with little significant homology to known structures, the problem of predicting secondary and tertiary structure accurately enough for drug design applications is still insurmountable.[113] Error rates for the various secondary structure prediction approaches are usually greater than 40%.[114,115] However, several of the current methods can suggest families of possible secondary structures that may be useful for some applications (e.g. site-directed mutagenesis). Few predictions of complete secondary and tertiary structure have been reported. A realistic appraisal of the current state of the art is represented by Cohen et al.'s ambitious prediction[116] of the core tertiary structure of Interleukin-2 prior to its determination by X-ray crystallography;[117] while the prediction had several key features correct, it was too inaccurate to be useful for drug design[118]–even small errors in the placement of secondary and tertiary structure can lead to major errors in the complete model.

VI. Modeling Drug–Receptor Interactions

The major interactions involved in drug–receptor binding are electrostatic (including hydrogen bonding), dispersion or van der Waals, and hydrophobic.[119] Hydrophobic interactions usually provide the major driving force for binding, while hydrogen-bonding and electrostatic interactions primarily provide specificity and often add little to the free energy of binding.[120-122] Drug–receptor "docking" is typically done interactively with molecular surface displays (e.g. "extra radius" surface) used to guide the fit, based on hydrophobic or electrostatic potential color coding. Since it is difficult to hit a moving target, the binding site is usually treated as completely rigid initially, while the conformation of the ligand is adjusted interactively. Recent systems are fast enough to provide real-time energy calculations while docking (future systems may use this information to provide feedback and prevent steric collisions or high-energy conformations). High-energy contacts can be shown with color-coded vectors.[123] Interactive docking thus alternates between continuous motion, possibly with real-time updates of the interaction energy if fast hardware is available, and periodic cycles of energy minimization to clean up the visual fit. A simple feedback approach that scales the dial (or joystick) response based on the instantaneous derivative of the interaction energy facilitates docking.[124] If the user moves uphill in energy, the system resists the motion, but if the user is moving in a favorable direction, the system encourages the motion by increasing responsiveness, so the docking tends to follow the path of least resistance in a sort of interactive energy minimization. Finally, energy minimization of the entire complex, where all atoms are allowed to relax, provides a good indication of the plausibility of the model and a rough estimate of the relative interaction enthalpy of the candidate drug. Ionic interactions and hydrogen bond energies are usually overestimated in a typical calculation due to the omission of solvent hydrogen-bonding competition; these effects are treated properly in the free energy perturbation theory method described below.

Conventional energy minimization with this many degrees of freedom is easily trapped in local minima and can give deceptive results; energy minimization rarely produces a structure that is significantly different from the starting coordinates. Molecular dynamics simulations as short as 10 ps are much better at escaping local minima and can give much lower energy structures; a good strategy is to begin with a short dynamics run and follow it with energy minimization. Such short dynamics simulations contain no meaningful information about the actual motions or dynamics of the structure (up to 30 ps may be required just for thermal equilibration); they simply provide a more efficient method of energy minimization and a good indication of the stability of the model (poor models tend to fly apart very quickly).

Multiple binding modes are often possible, as shown by the X-ray structure of an elastase-product complex in which the ligand is bound backwards to the established mode of productive binding.[125] It can be very difficult with interactive methods to find the most likely binding

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mode candidates. Naruto et al.[126] used a systematic search procedure to find chymotrypsin tetrahedral intermediate conformers given a covalent bond linking the ligand with the site. DesJarlais et al.[127] developed a general docking method for conformationally flexible ligands based on a fast sphere-matching algorithm by docking each rigid fragment of the ligand (fragments between rotatable bonds) independently.

A major problem with all design approaches is our current lack of ability to calculate even a qualitatively accurate estimate of the free energy of binding between two molecules in aqueous solution. An important advance in modeling ligand–receptor interactions is the recent application of free energy perturbation methods.[129,130] This takes advantage of the properties of a thermodynamic cycle to simulate a physical process which is very difficult to calculate (the transfer of a drug from solution into a receptor binding site, compared with the transfer of its analogue) by an equivalent nonphysical process (the "mutation" of a drug into its analogue, performed both in solution and in the binding site) which is relatively easy to calculate. This "mutation" is carried out by gradually changing the parameters of the initial drug molecule to the parameters of the final drug molecule during a molecular dynamics simulation, which is performed once in "solution", usually in a box of several hundred water molecules, and again in the macromolecule. The simulation starts with 100% initial drug character and ends with 100% final drug character; intermediate steps in the simulation have nonphysical hybrid drug molecules. Molecular dynamics generates a statistical mechanical ensemble average at each point along the simulation as the properties of the initial molecule are varied. Such simulations require large amounts of supercomputer time.

Wong and McCammon[131] described the calculation of the free energy difference of binding benzamidine vs *p*-fluorobenzamide to trypsin, while Bash et al.[132] reported calculations on free energy of binding differences for several thermolysin inhibitors and for a single thermolysin inhibitor to different mutant thermolysins. Both simulations were accurate to within less than 1 kcal of the experimental value. These results demonstrated how important the role of differential solvation can be in determining binding-affinity differences. It is not clear yet how large a difference between molecules can be simulated; all drug–receptor simulations so far have involved conservative single atom replacements, although Singh et al.[133] found excellent results with changes in entire amino acid side chains for calculating differences in solvation free energy. Free-energy perturbation methods are gradually becoming available in several molecular modeling systems, although this is still a frontier research area and it is not clear what the best approaches are or how long a simulation must be run to ensure statistically significant results.

Free energy perturbation methods offer the exciting possibility of calculating accurate differences in binding free energies between related ligands, which could make it possible to predict the binding affinity of new compounds prior to synthesis. Merz and Kollman[188] recently demonstrated the predictive ability of the approach by estimating the $\Delta(\Delta G)$ of thermolysin binding to a new inhibitor. However, recent work[189,190] has pointed out that it is extremely difficult to verify when a simulation has converged and has shown that some of the early reports were rather optimistic and tended to overestimate the precision with which $\Delta(\Delta G)$ was calculated. It is now clear that additional basic research is necessary before the method can be routinely applied and yield quantitatively reliable results. Current results suggest that $\Delta(\Delta G)$ for ligand–macromolecule binding can be calculated to within ±1.5–2 kcal/mol (equivalent to about a factor of 10–30 in binding affinity). Van Gunsteren[189], and Pearlman and Kollman[190] reviewed problems and pitfalls of the approach recently.

VII. Design

In the past, drugs were designed with an almost total naivete from the point of view of the molecular mechanisms of the underlying molecular machinery involved. The recent developments in Molecular Biology have clearly revealed the critical importance of three-dimensionality (3D) in molecular recognition and discrimination aspects. Even when the 3D features of the biological proteins involved were not known, drug design conducted along with this line emerged as an important aim and stimulated the development of some of the techniques mentioned in paragraph IV. Examples of lead molecules conceived in this way have been regularly reviewed,[1,5,164] and it is beyond the scope of this article to review all the excellent contributions that were made in this perspective.

As far as direct drug design is concerned, the ability to model both small organic molecules and macromolecules in the same system is critical; several of the systems currently available were originally designed for handling the regular, repeating polymeric structure of proteins and nucleic acids and deal rather poorly with the more arbitrary structures found in small organic molecules. Others were initially designed for modeling small molecules and do not handle macromolecular structures well. Few systems come close to offering the best of macromolecular and small-molecule modeling in an integrated system, providing the ability to interactively design and build potential ligands directly into a macromolecular receptor binding site.

Computer graphics enables us to qualitatively visualize drug–receptor interactions and molecular mechanics can provide rough estimates of the interaction energy, which allow us to design molecules that are apparently complementary to a binding site. For close analogues this can be sufficient to both rationalize the relative activities of a series of analogues and design new, closely related analogues; several excellent examples of this approach have been reported.[96,97,134] An integrated approach[135] combining molecular modeling with QSAR has proven to be especially powerful for this application, since the QSAR can help differentiate between different possible binding modes. We have much less experience in the de novo design of novel molecules (without a lead compound in an X-ray structure with its receptor). The designs by Beddell et al. of 2,3-diphosphoglycerate mimics[136] and antisickling com-

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pounds[137] based on the hemoglobin X-ray structure are still some of the best examples of this approach, despite the fact that most of this work was done with wire models! The only other reported successful example of de novo design using computer modeling methods is the design of phospholipase A_2 inhibitors by Ripka et al.[138]

All of the approaches we have described so far are analytical and oriented toward modeling known structures. Where do the structures of novel candidate drugs come from? Actual molecular structure design is still a formidable challenge dependent on the creativity, ingenuity, and experience of the medicinal chemist. Goodford developed a simple molecular mechanics based approach for calculating optimal ligand atom locations in a binding site, which is an important first step.[139] The method is based on calculating the interaction energy for each of a variety of probes (e.g. hydroxyl oxygen, carbonyl oxygen, carboxyl oxygen, amide nitrogen, amine nitrogen, etc.) at each point on a three-dimensional grid superimposed on the binding site. The grid is then contoured by energy, and the resulting contours are graphically displayed (as color-coded contour maps or dot clouds) in the binding site. The contours indicate predicted "hot spots" where a ligand atom of a given type should prefer to bind. Unfortunately it is usually very difficult to connect each of these "hot spots" together into a synthetically accessible molecule in a low-energy conformation, but the method does provide useful visual clues for structure design.

Current design techniques combine Goodford's (or related methods) with the other previously described interactive methods, where the investigator fits a variety of organic fragments in a trial and error fashion into the site, attempting to eventually combine the fragments into a complete molecule. The best approach is usually to design and build the developing ligand piece by piece in the binding site by combining preformed fragments from a library of different ring systems and functional groups and/or with CONCORD.[28] Small molecules can be built rapidly this way, and the resulting structures are usually accurate enough for initial qualitative "docking" into the site model. This is where good interactive software design and a well-thought-out user interface are especially important, since the modeler will spend much of his time in this stage trying out new ideas. Although it seems likely that all the information required for the design of an optimal ligand is present in the high-resolution structure of the receptor site, no systematic approaches exist yet for complete de novo design. The sphere-matching flexible ligand docking approach of DesJarlais et al.[127] or a 3D pharmacophore search over a 3D database[30,33,140] may eventually be able to achieve this, by docking fragments from a large library and then combining the fragments into complete molecules.

Very recently Dean and Colleagues[165-168] have published exploratory investigations concerning the possibility of automated site-directed drug design. The aim is to conceive appropriate algorithms and to construct a knowledge base for the automatic construction of novel ligands to fit specified binding sites.

VIII. Molecular Modeling Software

Major currently available academic and commercial molecular modeling software systems are listed below, along with their major functions. Currently, available computer (PC) programs have limited functionality for medicinal chemistry applications; they have not been included in this paper. Gerson[175] and Sadek[187] recently compiled reviews of PC software available for basic molecular modeling applications.

Tripos[141] has developed an excellent PC (IBM PC or Apple Macintosh II) interface to the host software (running on a superminicomputer or workstation) using the PC's local processing to provide real-time graphics display and manipulation of up to 100–200 atoms. This approach, which is now appearing in an increasing number of modeling packages, takes advantage of the inexpensive, fast graphics performance of the latest generation of PC's for display of small to medium-sized molecules, but retains the full functionality of the host software on a larger computer.

Program	Functions[a]
AMBER[36]	M, MM, MD, FE
BIOGRAF[142]	G, S, M, CA, MM, MD, MO
CHEM-X[31]	G, S, M, CA, MM, STAT, MO
CONCORD[28]	S
DISGEO[53]	DG
DISMAN[54]	DG
DSPACE[76]	DG
EMBED[78]	DG
FRODO[143,144]	G, M
GRID[139,145]	PR
GROMOS[146]	M, MM, MD, FE
INSIGHT/DISCOVER/DELPHI[41]	G, S, M, CA, MM, MD, MO
MACROMODEL[147,148,157]	G, S, M, CA, MM, MD, MO
MIDAS[149,150]	G, M
MM2[34]	MM, CA
MOGLI[151]	G, S, M
QUANTA/CHARMM[39,152]	G, S, M, CA, MM, MD, FE, PR, STAT, MO
SYBYL/ALCHEMY/NITRO[141]	G, S, M, CA, MM, MD, STAT, MO

[a] G graphic display and manipulation
S Small molecule structure building
M Macromolecules structure building
CA Conformational analysis facilities
MM Molecular mechanics
MD Molecular dynamics
FE Free energy perturbation methods
DG Distance geometry
PR Probe interaction energies
STAT Statistical tools
MO Molecular orbital methods from QCPE

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IX. Perspective

Crystallographers pioneered techniques to visualize, scrutinize, and manipulate three-dimensional molecular models. For example, the ORTEP[153] program plots crystal structure illustrations. ORTEP is still widely valued, in particular to add the third-dimension perspective to molecular structure representations. Another early example of a macromolecular graphics system is FRODO,[143,144] a software program used to facilitate electron density fitting experiments and to display and examine protein structures.

Quite independently, early attempts to incorporate computational chemistry methods to study the properties of molecules of biological interest have appeared in software such as, for example, AMBER,[36] CHARMM,[39] PCILO,[63,64] MM2,[34] and CAMSEQ.[154]

It was not until later, however, that molecular modeling graphics systems emerged from the combination of the above techniques and methods. With the addition of a conformational dimension to support structure–activity studies, the medicinal chemist was progressively offered an expanding arsenal of tools to assist and enhance drug design attempts. As outlined in this review, there is now an ample choice of molecular modeling software and methods available to the medicinal chemist.

Initial modeling software packages have been designed to provide methods dedicated either to small organic molecule or macromolecular modeling applications. Recently, progress has been made in combining both applications in a single package. However, a better integration of these two aspects is still needed to improve compatibility and enhance user interaction. In addition, future developments should benefit from a concerted combination of strengths in specific techniques and methodologies, particularly when addressing the increasing number of applications for the study of the interactions between small organic molecules and macromolecules.

Recent evolution in hardware and software technologies has made possible both implementation and development of methods (e.g., molecular dynamics, real-time manipulation of colored solid-shaded images for macromolecules) that were prohibitive not so long ago. Simultaneously, software packages have progressed to take advantage of powerful state-of-the-art features (e.g., windowing, menu-driven systems, command language syntax). However, the desirable user-friendly interface has been somewhat overlooked in this evolutionary process, and modeling software can appear rather complex and cumbersome to occasional users. We hope that future developments will address this issue.

Advances in molecular modeling have been impressive over the last years. Major milestones in software and hardware technologies have been accomplished and future prospects in this rapidly evolving arena look very promising. Current efforts to develop and integrate methods and techniques to assist and enhance drug design studies should lead to even higher levels of computer automation, rationalization, quantification, and, eventually, de novo design of novel molecules.

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