Modelling mutations and homologous proteins

Andrej Šali

The Rockefeller University, New York, USA

A protein sequence with at least 40% identity to a known structure can now be modelled automatically, with an accuracy approaching that of a low-resolution X-ray structure or a medium-resolution nuclear magnetic resonance structure. In general, these models have good stereochemistry and an overall structural accuracy that is as high as the similarity between the template and the actual structure being predicted. As a result, the number of sequences that can be modelled is an order of magnitude larger than the number of experimentally determined protein structures. In addition, evaluation techniques are available that can estimate errors in different regions of the model. Thus, the number of applications where homology modelling is proving useful is growing rapidly.

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Introduction

Comparative or homology protein modelling uses experimentally determined protein structures (templates) to predict the conformation of another protein with a similar amino acid sequence (target) [1-5,6-9]. Comparative modelling is possible because a small change in the sequence usually results in a small change in the three-dimensional structure [10-14,15-16]. Although considerable progress has been made in ab initio structure prediction [17-19,20,21], comparative modelling remains the only modelling method that can provide models with a root mean square (rms) error lower than 2 Å. In general, the best comparative techniques can produce models with good stereochemistry and overall structural accuracy that is as high as the similarity between the template and the actual target structure. Thus, the comparative method can result in models with a main-chain rms error as low as 1 Å for 90% of the main-chain residues, if a sequence is at least 40% identical to one or more of the templates [22,23]. In this range of sequence similarity, the alignment is mostly straightforward to construct, few gaps exist and structural differences between the proteins are usually limited to loops and side chains. When sequence identity is between 30% and 40%, the structural differences become larger, and the gaps in the alignment are more frequent and longer. As a result, the main-chain rms error rises to ~1.5 Å for ~80% of the residues. The rest of the residues are modelled with large errors because the methods generally cannot model structural distortions and rigid body shifts, and they cannot recover from misalignments. In such situations, model evaluation methods can be used to identify the inaccurately modelled regions of a protein. When sequence identity drops below 30%, the main problem becomes the identification of related templates and their alignment with the sequence to be modelled.

Despite these limitations, comparative modelling is useful because about one-third of known sequences appear to be related to at least one known structure [24,25]. Because only ~2000 of the about 100,000 known protein sequences have had their structures determined experimentally, the number of sequences that can be modelled relatively accurately is an order of magnitude larger than the number of experimentally determined protein structures. Furthermore, the usefulness of comparative modelling is steadily increasing because genome projects are producing more sequences and because novel protein folds are being determined experimentally.

In the early eighties, manual comparative modelling [26,27] was facilitated by the manipulations of protein molecules on the graphics terminal [3,28], which was made possible by computer programs such as FRODO [29]. This approach was later improved by the introduction of largely automated modelling algorithms that could use several known structures to model the unknown member of the family [30,31]. This group of methods is based on the assembly of the model from a few core regions, and loops and side chains, which are obtained from dissected related structures [1,3,28]. Another group of comparative methods relies on the approximate positions of conserved atoms from the templates to calculate the coordinates of other atoms, using a database of short segments of protein structure, energy or geometry rules, or some combination of these criteria [32-35]. A third group of comparative methods is based on the satisfaction of spatial restraints obtained from the alignment of the target sequence with homologous templates of known structure [2,36,37].

Abbreviations

NMR—nuclear magnetic resonance; rms—root mean square; WWW—World-Wide Web.
And a final group of methods, which is not covered in this review, consists of recognition of the native fold by threading a target sequence through each fold in a database of all known folds [38,39]. This can be seen as a first step towards modelling sequences that are only distantly related to the known protein structures [40–50,51*,52*,53]. In addition to methods for modelling the whole fold, numerous other techniques for prediction of loops and side chains on a given framework have also been described. These methods can often be used in combination with each other and with comparative modelling techniques.

This review is organized in terms of the main stages that are shared by all comparative modelling methods. The first step is always to align the target sequence with all the related proteins whose three-dimensional structures are known. In the second step, the alignment and the structures are used to build a model for the target sequence. The main difference between the comparative methods is in how the alignment is used to get the three-dimensional model. In the third step, the model is evaluated and, if necessary, the alignment and model building are repeated until a satisfactory model is obtained. For each of the three steps, I first provide a brief historical overview and then describe in more detail the latest developments published since 1993.

Finding and aligning template structures with the target sequence

The first task in comparative modelling is to identify all protein structures related to the target sequence, some of which will be used as templates. This is greatly facilitated by databases of protein sequences and structures and software for scanning those databases (for reviews, see [6,5,54*,55–57]). At present, the probability is ~30% that a sequence picked randomly from a sequence database has at least 25% sequence identity to at least one known structure [55*].

The target sequence can be searched against sequence databases, such as Protein Identification Resource (PIR) [58], GENBANK [59], SWISS-PROT [60], or EMBO nucleotide sequences database [61], and/or structure databases such as the Brookhaven Protein Database [62] and SCOP [63*]. The most popular programs, including FASTA [64] and BLAST [65], compare the target sequence with each sequence in a database. Program MODELER (see below), which implements all the stages in comparative modelling [5], can also automatically search for proteins with known three-dimensional structure that are related to a given sequence. The sensitivity of the search can be improved if the target sequence is aligned against sequence templates constructed from multiply aligned sequences [66,67*,68,69].

Additional sensitivity in detecting remote relationships is gained when structural information about potential homologues is used. Typically, the target sequence is matched against a library of three-dimensional profiles or threaded through a library of three-dimensional folds [45–47,55,70,71*]. These more sensitive fold identification techniques are especially useful for finding significant structural relationships when sequence identity drops below 30%.

Once all the structures related to the target sequence are identified, the second task is to prepare a multiple alignment of the target sequence with all the potential template structures. The alignment can frequently be improved if other sequences from the same family are also aligned at the same time. This additional effort is often useful because the quality of the alignment is the single most important factor determining the accuracy of the three-dimensional model. In principle, most sequence-alignment and structure-comparison methods can be used, but in practice it is frequently necessary to edit manually the positions of insertions and deletions to ensure that they occur in a reasonable structural context (e.g. not in the middle of a helix). Comparison methods are not reviewed here (for reviews, see [6,54*,56,72]).

Although profile matching and threading techniques are relatively successful in identifying related folds, they appear to be somewhat less successful in generating correct alignments. This limits the use of alignments from threading because comparative modelling cannot, at present, recover from an incorrect alignment. At 30% sequence identity, the fraction of correctly aligned residues is ~80%, but this number drops sharply with further decrease in sequence similarity [73]. This implies that reasonable homology models can be obtained only for sequences that have more than 30% identity to at least one known structure. With such a high similarity, the potential template structures can be almost always identified and aligned using the simplest sequence based searches and alignment techniques. Sequence identity of at least 30% almost guarantees that two chains longer than 50 residues will have related three-dimensional structures [12].

The power of the databases to address various questions is greatly enhanced when relationships between the proteins are established. Several collections of alignments of protein structures have been published that facilitate both the development and the use of comparative modelling techniques [74–78,79*].

Once a multiple alignment is constructed, a matrix of pairwise sequence identities is usually calculated and employed to construct a phyletic tree that expresses the relationships among the proteins in the family [80]. All significantly different structures in the cluster that contains the target sequence are usually used as templates in subsequent model building [81]. Some methods allow short segments of known structure, such as loops [32], to be added to the alignment at this stage [5].

Model building

Modelling by assembly of rigid bodies

The first approach used for comparative modelling was to assemble a model from a small number of rigid
bodies obtained from the aligned protein structures [1,3,26-28,30,31,82-85]. For example, in the computer program COMPOSER [30,31], three types of rigid body are used to build the model. Each individual rigid body of the model is selected as the best rigid body from the corresponding set of all possible rigid bodies. These sets are the following: first, for a conserved core region, the equivalent segments of contiguous main-chain atoms from homologous structures; second, for a loop, the equivalent loops from homologous proteins and loops satisfying certain geometric criteria from other structures; and finally, for a side chain, the equivalent side chains from homologous structures, as well as the most likely side-chain conformations found in proteins in general. These rigid bodies are assembled on the framework, which is defined as the average Cα atoms in the conserved regions of the fold.

Recently, Srinivasan and Blundell [23] have extensively evaluated comparative modelling by rigid body assembly. They found that the accuracy of a model can be somewhat increased when more than one template structure is used to construct the framework and when the templates are averaged into the framework using weights corresponding to their sequence similarities to the target sequence. For example, differences between the modelled and X-ray structures of the modelled protein may be slightly smaller than the differences between the X-ray structures of the modelled protein and the homologues used to build the model. Possible future improvements of modelling by rigid body assembly include incorporation of rigid body shifts, such as the relative shifts in the packing of α-helices [86].

Peitsch and Jongeneel [87] described an automated approach to homology modelling, similar to that of Blundell and co-workers [30,86]. They applied their approach to model the CD40 ligand [87].

Kajihara et al. [85] constructed a three-dimensional model of bovine pancreatic B-trypsin from four parts corresponding to each of its exons. These four building blocks were obtained as the most similar regions found in four other serine proteases with known three-dimensional structure. The model was then refined by molecular dynamics simulation. In agreement with [23], it was shown that this 'chimaera' approach is better than using only a single template structure.

Modelling by segment matching or coordinate reconstruction

The build-up procedure constructs the three-dimensional model by assembling short segments of the structure. The segments were originally generated and assembled according to the energetic criteria [88]. The use of this idea in comparative modelling was facilitated by the finding that only ~100 different hexamers can be joined together to cover 99% of the residues in proteins [33]. This paved the way to a new approach to comparative modelling, in which a subset of atomic positions in the template is used to identify short segments in all known protein structures that fit on the guiding positions. The short segments are then assembled into the complete model. For example, Claessens et al. [34] developed a method for modelling the backbone with 'spare parts', short segments of varying length from other structures that were identified by matching the guiding Cα positions. Other similar backbone reconstruction procedures have been described [89-91]. A more general segment match modelling by Levitt [35] is guided by the positions of some atoms (usually Cα atoms) to find the matching segments in the representative database of all known protein structures. This method can construct both side-chain and main-chain atoms, and it can also model insertions and deletions.

Many methods for constructing coordinates of missing atoms from the positions of guiding atoms rely on geometric or energetic criteria and possibly on a conformational search, instead of depending on a database of segments [92-96,97]. Usually, the guiding positions are Cα atoms of a subset of residues, and either main-chain or full-atom models are constructed. These methods can be applied to comparative modelling when homologous structures are used as the source of the guiding positions and when combined with the loop and side-chain construction algorithms [89,90,98].

Even the class of loop construction methods based on finding suitable fragments in the database of known structures [32] can be seen as a segment matching or coordinate reconstruction method. The same is true for some side-chain modelling methods [99].

Payne [96] used Cα coordinates to reconstruct complete backbone coordinates and side-chain directions. A potential of mean force, derived from a database of protein structures was employed to orient the peptide groups around axes connecting successive Cα atoms. Because terms of the scoring function were local in nature, a dynamic programming procedure could be used for optimization.

Van Gelder et al. [97] have presented a new method to build a complete protein structure from Cα coordinates. The first step in this approach is to generate an approximate backbone using geometrical criteria only. In the second step, the backbone is refined and side chains are positioned using exhaustive molecular dynamics simulation. These authors used the method to generate full-atom models of two proteins from their low-resolution Cα traces.

Modelling by satisfaction of spatial restraints

It is important to distinguish between constraints and restraints. Constraints restrict a spatial feature, such as a distance between two atoms, to a particular single value, whereas restraints allow a wider range of values, possibly with varying probabilities.

Srinivasan et al. [36] described a three-dimensional model of bungarotoxin that was obtained through the
use of distance geometry to satisfy main-chain distance constraints extracted from the cobratoxin structure and a low-resolution structure of bungarotoxin. A general method for modelling by optimization of spatial restraints obtained from the alignment of the target sequence with homologous templates of known structure was proposed by Sali and Blundell [2,100]. An elegant distance geometry approach for constructing all-atom models from lower and upper bounds on distances and dihedral angles was described in detail by Havel and Snow [37]. Other methods based on satisfaction of main-chain distance restraints by molecular dynamics were reported by Fujiyoshi-Yoneda et al. [101] and Engh et al. [102]. A protein backbone has also been modelled by satisfying Cα-Cα contacts predicted by a neural network that relied on an alignment between the target sequence and a template structure [103].

Recently, Havel [104] has extended the earlier approach of Havel and Snow [37]. Lower and upper bounds on Cα-Cα distances, main-chain-side-chain distances, hydrogen bonds, and conserved dihedral angles were derived for Escherichia coli flavodoxin from four other flavodoxins; bounds were calculated for all distances and dihedral angles that had equivalent atoms in the template structures. The permitted range of values of a distance or a dihedral angle depended on the degree of structural variability at the corresponding position in the template structures. Distance geometry was used to obtain an ensemble of approximate three-dimensional models, which were then exhaustively refined by restrained molecular dynamics with simulated annealing in water.

Comparative modelling by optimization of a potential function constructed from a sequence alignment with related structures was described by Snow [105]. His model consists of Cα atoms that are restrained by a form of a Lennard–Jones potential. The position of the minimum of each Lennard–Jones term corresponds to a weighted average of equivalent distances in homologous structures and the depth of the minimum is inversely proportional to the variability among these distances. The ‘energy’ is minimized by a simulated-annealing procedure in the angle and dihedral angle space, followed by a conjugate gradient refinement in the Cartesian space. The method is tested by modelling rubredoxin on the basis of four other rubredoxin structures.

The method developed by Srinivasan et al. [106] uses a single template structure to obtain distance constraints on the target sequence. As in [37], constraints are derived for all pairs of atoms that have equivalent pairs in the template structure. Distance constraints are satisfied by a distance geometry program and a subsequent energy refinement. When the template and target sequences are similar, the target structure is also very similar to the template structure. Subsequently, the method has been improved by relaxing distance constraints on the target sequence outside the manually delineated structurally conserved regions [107*]. This relaxation facilitates three-dimensional embedding and energy minimization, and increases the rms between the template and the model, but it does not appear to increase the accuracy of the model beyond the similarity between the template and the actual structure of the target [107*].

Broekhust and Perham [108] have described an automated method for constructing a three-dimensional model of a sequence that is aligned with related structures. This method optimizes a relatively small number of spatial restraints that are judged to be important for the fold and/or function and thus more likely to be conserved in the family of proteins. These restraints act upon main-chain hydrogen bonds, attractive van der Waals contacts, and main-chain and side-chain dihedral angles. The optimization relies on the program X-PLOR [109] and consists of molecular dynamics with simulated annealing. The method has been applied to two domains from the dehydrogenase family.

I now focus on the modelling approach of Sali and Blundell [2,5,22*,79*,100,110*]. The question addressed is ‘What is the most probable structure for a certain sequence given its alignment with related structures?’ Our approach follows from the method for comparison of protein structures implemented in the program COMPARER [100,111] and was developed to use as many different types of data about the target sequence as possible. It is implemented in the computer program MODELLER (which is available by anonymous ftp from tammy.harvard.edu:pub/modeller and also as part of QUANTA [MSI, Burlington, Massachusetts, USA; E-mail: jcollins@msi.com]). The input to the program is an alignment of the target sequence with related known three-dimensional structures. The output, obtained without any user intervention, is a three-dimensional model for the target sequence containing all main-chain and side-chain heavy atoms. First, MODELLER derives many distance and dihedral angle restraints on the target sequence from its alignment with template three-dimensional structures. Spatial restraints on the target sequence are obtained from the statistical analysis of the relationships between various features of protein structure. A database of 105 family alignments, including 416 proteins with known three-dimensional structures, was constructed [79*] to obtain the tables quantifying the relationships, such as those between two equivalent Cα-Cα distances, or between equivalent main-chain dihedral angles from two related proteins. These relationships were expressed as conditional probability distributions and can be used directly as spatial restraints. For example, probabilities for different values of the main-chain dihedral angles are calculated from the type of a residue considered, from main-chain conformation of an equivalent residue, and from sequence similarity between the two proteins. An important difference from the other methods discussed in this section is that the spatial restraints are obtained empirically from a database and are not guessed. Next, the homology-derived restraints and energy terms enforcing proper stereochemistry [112] are combined into an objective function. Finally, the model is obtained by
Modelling of loops

Loops can be calculated by searching a structure database for segments that fit on fixed endpoints [32], by conformational search with an optimal energy minimization [125–127], or by a combination of these approaches [128,129]. Many different implementations of the basic techniques have been described [32,125–137,138**,139–144,145**,146,147**,148,149].

Collura and colleagues [141,142] used Monte Carlo and simulated-annealing algorithms to optimize a united atom energy function for a loop that spans given anchor regions. The energy function consists of non-bonded atomic interactions and a harmonic potential applied to terminal residues to force the loop closure. The degrees of freedom include only the main-chain and side-chain dihedral angles, excluding the α dihedral angle. The optimization started from a completely extended conformation. For loops seven residues long, the average rms error was 1 Å for main-chain atoms and 2.3 Å for all heavy atoms.

Rao and Teeter [139], who also relied on a united atom model, optimized the energy of a single turn by a molecular dynamics procedure, as implemented in both AMBER [150] and X-PLOR [139]. An incorrect starting conformation changed into approximately correct conformation, as seen in the refined X-ray structure. It was shown that, in contrast to the original model, the predicted turn conformation refined with the X-ray data in fewer cycles, without any manual intervention, and with better refinement statistics.

Zheng et al. [144] described a new method for loop closure that started with all bonds scaled so that a random starting conformation fitted on the anchor regions. The loop was then relaxed to its standard geometry. The predictions were enhanced by taking into account the protein environment of the loop. The method has been combined with multiple copy sampling to increase its efficiency by up to a factor of five [145**]. It has also been demonstrated that the variability in the predicted loop conformations can be used to estimate the accuracy of the models. In further development, the technique has been applied to model more than one loop at the same time [151**]. As a result, more accurate predictions were invariably obtained. The rms errors for 5–7 residue loops ranged from 0.6–1.7 Å for backbone atoms.

Srinivasan and Blundell [23] have described a collar extension idea for modelling loops. This relies on an equivalent loop from a homologue that differs by one or two residues in length. The equivalent part is copied from the template to the target and the remaining short gap is modelled by the database search approach, as described above [32].

Topham et al. [136] improved selection of the correct loop from an ensemble of candidate loops that already fit relatively well on the two anchor regions. This was achieved using three-dimensional profiles; candidate loops were ranked by a scoring function based on three-dimensional profiles that evaluated the compatibility between each residue in the target sequence and the environment implied by the structure of a candidate loop. The criteria included in the three-dimensional profiles were main-chain conformation, solvent accessibility, hydrogen bonding, disulfide bonding, and α-helix conformation.

Fidelis et al. [138**,139] have compared database and conformational search methods for loop modelling. They show that little correlation exists between the similarity in the anchor and loop regions of two segments and that the database of segments is sparse for segments longer than eight residues. The systematic search procedure can generate almost all structures of short segments in proteins and is thus the preferred method for modelling loops.

A new type of method, based on the self-consistent field approximation that was previously applied to side-chain modelling [152**], has been described by Kochl and Delarue [153**]. The method uses a database search scheme to generate possible main-chain fragments for modelling loops [32] and a rotamer library to define possible side-chain conformations [154]. It then iteratively refines the probabilities that the backbone and side chain of each residue correspond to database fragment j and rotamer k, respectively. Each residue experiences the average of all possible environments. The energy function includes only van der Waals terms, but can clearly be extended to include other terms, such as hydrogen bonds and solvation. The method usually converges to a single answer very close to one of the template structures. The self-consistent field method can be seen as the way to select one of the segments with which the target sequence is aligned. In principle, the method could be used to model whole structures.

Sudarsanan et al. [147**] have described a method for modelling loops on a given framework. Of the order
of 10000 loop conformations are generated for each loop. Starting from the amino terminus of the loop, conformations are generated by assigning randomly selected pairs of $\Phi_{i+1}, \Psi_i$ for each dimer, using standard geometry and trans peptide configuration. The predicted loop is the conformation that maximally satisfies the loop closure condition and does not have any atom–atom overlaps. Additional filters, such as disulphide bonds, can easily be imposed on the construction of the loops.

The conformational properties of tight two-residue $\beta$-turns have been examined by analyzing 3899 examples in 205 protein chains [155*] and by empirical energy function calculations [156*]. It was shown that the conformation of such turns is determined by the twist of the $\beta$-sheet and a local electrostatic effect, and that the conformation can be modelled well when the rest of the protein and crystal water molecules are included in the calculations [156*]. Borchert et al. [149] modelled a seven-residue loop in a monomeric triosephosphate isomerase fold using program ICM, a general tool for conformational search in the dihedral angle space guided by a detailed energy function [157**]. The predicted loop had an rms of only 1.2 Å for the 28 main-chain atoms. These successes are in agreement with the analysis of Fidelis et al. [138**, who showed that loops shorter than seven residues can often be modelled correctly. This probably the result of a relatively small number of loop conformations consistent with given anchor regions. Database search methods do not work well for loops longer than eight residues because the database is not likely to contain an example of a loop being modelled and because the correlation between the similarity of the anchor and loop regions is very weak [140], so that even if the correct conformation were in the database, it could not be easily identified. Possible reasons for failure of conformational search methods to model loops longer than eight residues include insufficient accuracy of the energy function, inadequate sampling of the phase space, and failure to include enough of the loop environment in the optimization. Fortunately, few insertions in a family of homologous proteins are longer than eight residues [14,158,159].

Modelling of side chains
As for loops, side-chain conformation has been predicted from similar structures, from proteins in general, and from steric or energy considerations [31,83,98,99,154,160–169,170**,171,172,173*–176*,177,178,179**,180*–182*]. The geometry of disulphide bridges has been modelled from disulphide bridges in protein structures in general [183–187,188] and from equivalent disulphide bridges in related structures [79*]. For information on modelling the stability and conformation of point mutations by free energy perturbation simulations, the reader is referred elsewhere [189–192].

Dunbrack and Karplus [169] have developed a backbone-dependent rotamer library for amino acid side chains, using it to construct side-chain conformations from main-chain coordinates. They found significant correlations between side-chain dihedral angle probabilities and backbone $\Phi$, $\Psi$ values. These correlations go beyond the dependence of side-chain conformation on the secondary structure [164]. This automated method first places the side chains according to the rotamer library, and then removes steric clashes by energy minimization. It is also demonstrated that simple arguments based on conformational analysis can account for many features of the observed dependence of the side-chain rotamers on the backbone [170**].

Eisenmenger et al. [193*] used program ICM [157**] to model side chains. Each side chain was configured in the environment of only the backbone atoms by a systematic search procedure combined with extensive local energy minimization of van der Waals, hydrogen-bond, torsional, and tether terms. Tests using main-chain atoms or both the main-chain and remaining side-chain atoms in the energy evaluations established the dominance of the main-chain contribution. The final model is obtained by a full energy refinement of the structure.

Tanimura et al. [181*] predicted side-chain conformations on a given backbone by a conformational search procedure relying either on side-chain–side-chain interactions or side-chain–main-chain interactions. In agreement with [193*], removal of side-chain–side-chain interactions did not cause a large decrease in the prediction accuracy. Even so, the model having only side-chain–side-chain interactions still retained a significant level of accuracy. These results suggest that the two classes of interaction are consistent with each other and work in harmony to stabilize native conformations [194].

Wilson et al. [171] randomly picked local sites of adjacent side chains throughout the protein and evaluated all combinations of side-chain rotamers within each site using a molecular mechanics force field enhanced by the inclusion of a solvation term. At each site, the lowest energy combination of side chains is identified and added onto the fixed backbone. The procedure is repeated until side-chain conformations converge. The robustness of the method is evaluated by perturbing the backbone coordinates. The rms of predicted side chains rose from 1.3 Å in a test case with the correct backbone to 2.7 Å in one with <35% identity.

Vriend et al. [99*] have introduced SCAN3D, a new database system for integrated sequence–structure analysis. Site–dependent, side-chain rotamer distributions are obtained by extracting short segments with a given main-chain conformation from a database of protein structures. These rotamer distributions are then used in side-chain modelling. In a separate analysis, a set of predictive rules was derived that relied on the site–dependent rotamers and hydrogen-bonding criterion to explain 85% of point mutations currently available [173*].

Cregut et al. [174*] have tested three methods for side-chain prediction. The methods included molecular mechanics conformational search, the use of a rotamer database, and a combination of these two methods. It was
shown that the rotamer-based method is more efficient and that energy minimization before rotamer selection does not afford clearly improved predictions. It was also demonstrated that implicit solvation terms improve the predictions and that most errors can be identified by a combination of evaluation criteria, including solvation energy, rms deviations, $\chi_1$ angle distributions, and hydrogen bonds.

Laughton [175*] has compared the local environment of each residue whose side chain is to be predicted with a database of local environments for the same residue type constructed from an analysis of high-resolution protein structures. Local environments are described in terms of the residue type, location of the residue and of neighboring residues that interact with the given side chain. The best few matches are input into a Monte Carlo procedure, which gives the final model by removing the steric clashes in the structure. In further development, the AMBER program has been used to explore a variety of simulated-annealing protocols and modifications of the united-atom force field for side-chain modelling [176*]. In this work, the modelling problems are generated by defining a percentage of side chains in a given X-ray structure as undefined.

The use of the dead-end elimination theorem in side-chain modelling (which was discussed previously [167]) has been re-examined [168]. The original theorem was meant to identify rotamers and pairs of rotamers that could not be part of the global energy minimum. This would allow a large reduction in the search space, thus leading to a possibility of a deterministic search for the global energy minimum. It was shown that the dead-end elimination theorem was not correct for rotamer pairs. Yet, a "fuzzy" version of the theorem was proposed that still reduced the size of the search for the best conformation. It was used to help in modelling side-chain conformations given known backbone coordinates and a library of side-chain rotamers.

Koehl and Delarue [152*] have described a side-chain modelling method based on a rotamer library and a given backbone. It employs the self-consistent mean field approach. The method iteratively refines a conformational matrix of each side chain in a protein such that its current element $i, j$ at each cycle gives the probability that the corresponding side chain of rotamer $j$ adopts the conformation of its possible rotamer $i$. Each residue experiences the average of all possible environments, weighted by their respective probabilities. The final prediction corresponds to the rotamers with the highest probabilities. Estimates of the conformational entropy of side chains in the folded proteins are also given.

Lee [179*] has improved an earlier side-chain modelling method [177,178] by developing a new powerful optimization technique based on the self-consistent mean field approximation. Side-chains are built on a fixed backbone. The energetic of the system are described by Lennard-Jones and simple dihedral angle terms; no electrostatic or hydrogen-bonding terms are used. Side-chain dihedral angles are allowed to assume 33 different values. All possible conformations are initially set to have the same probability. The optimization then proceeds in the space of these probabilities, rather than in conformational space. The energy of each state for each dihedral angle is calculated as the mean field energy, given the current probabilities for all the other possible conformations. The probabilities are then recalculated using the Boltzmann distribution. These two steps are repeated many times at successively lower temperatures until the convergence in energy is achieved. To speed up the calculations, the mean field is not calculated exhaustively, but by Monte Carlo sampling of possible conformations. The method shares many features with that of Koehl and Delarue [152*], and they are both related to an approach to threading [43]. The robustness, speed, and size dependence of the new optimizer compare favourably with an earlier simulated-annealing method [178]. The method was used for calculating both protein thermostability differences and side-chain conformations. The calculated thermostability of the hydrophobic core mutants of $\lambda$ repressor compared very well with experimental data. Similarly, side-chain conformation was predicted reliably; for example, flavodoxin core side chains were modelled with an rms error of 1 Å when the crystallographically determined backbone of flavodoxin was used.

Although the solvation term is irrelevant for the modelling of core side chains, it is important for the modelling of exposed side chains [83,171,174*]. It has also been demonstrated that treating hydrogen bonds explicitly can significantly improve side-chain prediction [169,173*]. It appears that relatively fine sampling of the dihedral angle space is necessary to model some side chains; for example, Schrauber et al. [195] reported that 5–30% of the side chains, depending on the residue type, are substantially different (by >20°) from common rotameric states in highly resolved structures. Another point relevant for homology modelling is that the best side-chain conformation depends relatively strongly on the backbone conformation of the residue [99*,169,181*,195]. For example, the preferred rotamers can vary within the same secondary structure, with the changes in the $\Phi, \Psi$ dihedral angles as small as 20° [169]. Because these changes are smaller than the differences between closely related homologues, the prediction of the side-chain conformation generally cannot be uncoupled from backbone prediction. This partly explains why conformation of equivalent side chains in homologous structures is useful in side-chain modelling [5]. This is consistent with the X-ray structure of a variant of $\lambda$ repressor which reveals that the protein accommodates the potentially disruptive residues with shifts in its $\alpha$-helical arrangement and with only limited changes in side-chain orientations [196*].

Model evaluation

It is clearly necessary for a good model to have a low energy according to a molecular mechanics force
field, such as that of CHARMM [112]. Correspondingly, stereochemical tests have been incorporated in PROCHECK [197], a program that is based on the analysis of known protein structures [198]. The local criteria checked by PROCHECK include the distribution of main-chain and side-chain dihedral angles, and the geometry of bonds, angles, improper dihedral angles, planes and chiral centres. The seminal work by Novotny et al. [199,200] showed, however, that low molecular mechanics energy does not ensure that the model is correct. Thus, distributions of many spatial features have been compiled from high-resolution protein structures, and any large deviations from the most likely values have been interpreted as strong indicators of errors in the model. Such features include packing [201], creation of a hydrophobic core [202], residue and atomic solvent accessibilities [203–206,207], spatial distribution of charged groups [208], distribution of atom–atom distances [209], and main-chain hydrogen bonding [197].

Another group of methods for testing three-dimensional models, which implicitly takes into account many of the criteria listed above, involves three-dimensional profiles or threading. These methods evaluate the environment of each residue in a model with respect to the expected environment, as found in the high-resolution X-ray structures. The programs implementing this approach include 3DPROF [210], PROSII [211,212], THREADER [46], MATCHMAKER [45], and HARMONY [213]. In principle, an improvement in the accuracy of a model is possible by incorporating the quality criteria into a scoring function being optimized to derive the model in the first place.

Recently, protein modellers were challenged to model sequences without available three-dimensional structures and to submit them to the first 'Meeting on Critical Assessment of Techniques for Protein Structure Prediction' in Asilomar in December of 1994. At the same time, the three-dimensional structures were being determined by X-ray crystallography and NMR methods. Because the structures were only released at the meeting, it was possible to test the modelling methods objectively. The evaluation of comparative modelling can be summarized as follows [22]. In general, the best comparative techniques can produce models with good stereochemistry and overall structural accuracy that is as high as the similarity between the template and the actual target structures. The errors can be divided into four categories: first, errors in side-chain packing; second, distortions or shifts of a region that is aligned correctly with the templates (e.g., loops, helices and strands); third, distortions or shifts of a region that does not have an equivalent segment in any of the templates (e.g., inserted loops); and fourth, distortions or shifts of a region that is aligned incorrectly with the templates (e.g., loops and larger segments with low sequence identity to the templates). The last three of these errors are relatively infrequent when sequences with >40% identity to the templates are modelled. For example, in such a case, approximately 90% of the main-chain atoms are likely to be modelled with an rms error of ~1 Å [22]. Below 40% sequence identity, misalignments and insertions in the target sequence become the major problems. Insertions longer than about eight residues cannot be modelled accurately at this time, even when the alignment of the stem regions delimiting the insertion is correct. Most of the insertions shorter than eight residues also cannot be modelled successfully, primarily because the alignment of the inserted and neighbouring residues is frequently incorrect. If the length of an insertion can be extended enough to make the alignment of the delimiting stem regions reliable (but not too much, so that less than eight residues are inserted) the insertions can frequently be modelled successfully [138**,156*,157**]. In general, it can be expected that ~20% of the residues will be misaligned, and consequently incorrectly modelled, when the level of sequence identity between the target and templates is 30% [73].

To put errors into perspective, the differences among experimentally determined structures of the same protein can be compared. The 1 Å accuracy of main-chain atom positions corresponds to X-ray structures defined at a resolution of ~2.5 Å and with an R-factor of ~25% [214*] as well as to NMR structures determined from 10 inter-proton distance restraints per residue [215,216]. Similarly, differences between the highly refined X-ray and NMR structures of the same protein also tend to be ~1 Å [215]. Changes in the environment (e.g., crystal packing, solvent and ligands) can also have a significant effect on the structure [217]. Overall, homology modelling based on templates with >40% identity is almost as good, simply because the homologues at this level of similarity are likely to be as similar to each other as the structures for the same protein determined by different experimental techniques under different conditions. The caveat in modelling, however, is that some regions, mainly loops and side chains, have larger errors. Although such regions may have an important function, many applications in biology do not require high-resolution structures. For example, some binding sites may be located with the aid of low-resolution models [218].

We need a standardized, centralized, and comprehensive suite of model tests in order to benchmark existing methods and to aid in the development of new methods. Alignment as well as modelling of side chains, loops, and whole structures should be tested in an automated way. The first step in this direction is the 'Biotech Validation Suite for Protein Structures' accessible at the World-Wide Web (WWW) address http://www.embl-heidelberg.de:8400.

Conclusions

The existing comparative modelling techniques can be used in an automated way and without any subjective decisions, provided templates with at least 40% sequence identity are known; no significant improvement of such
models is achieved by subjective interventions. On the other hand, for sequences with sequence identity <40%, large errors in the alignment can sometimes be prevented by examining and editing the alignment manually. In general, models have good stereochromaticity and overall structural accuracy that is as high as the similarity between the template and the actual structure being predicted. As a result, the number of sequences that can be modelled is an order of magnitude larger than the number of experimentally determined protein structures, and the accuracy of a large fraction of these models is in many ways comparable to the accuracy of low-resolution X-ray structures and medium-resolution NMR structures. That is, >90% of main-chain atoms can be modelled with an accuracy of 1 Å, provided a template structure with at least 40% sequence identity is available. The errors in different regions of the model can be estimated by a variety of evaluation techniques.

Future improvements of comparative modelling should aim to model proteins with lower similarities to known structures, to increase the accuracy of the models, and to make modelling fully automated. The improvements are likely to include the simultaneous optimization of side-chain and backbone conformations in side-chain modelling, and simultaneous optimization of a loop and its environment in loop modelling. At the same time, better potential functions and possibly better optimizers are needed. The potential function should guide the model away from the templates towards the correct structure. An addition of atomic- or residue-based potentials of mean force to the homology-derived scoring function, such as that of MODELLER [5], could be one way of achieving this goal. To reduce the errors in the model stemming from the alignment errors, iterative changes in the alignment during the calculation of the model, perhaps similar to the threading techniques [45,46], are needed.

Even though comparative modelling needs significant improvements, it is already a mature technique that can be used to address many practical problems. Some successful predictions include identification of the heparin-binding site in the mouse mast cell tryptases [219], design of micromolar inhibitors of the malarial cysteine protease [220], prediction and conversion of substrate specificity of granzyme B [221], and solution of a molecular replacement problem in X-ray crystallography [222]. With the increase in the number of protein sequences and in the fraction of all folds that are known, comparative modelling will become even more useful in the future.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest


A comprehensive review of homology modelling and some other relevant techniques.


Comparative modelling by assembly of rigid bodies is reviewed.


A short review of comparative modelling.


Databases of protein structures and comparative modelling are reviewed.


Side-chain-side-chain contacts, accessibility, secondary structure, and rms deviations are compared within 607 pairs of proteins having similar three-dimensional structures. This should be useful in determining the invariants in protein structure and thus facilitate the design of better comparative modelling methods.


Analysis of conservation of coarse, medium, and fine-grain structural properties is performed over a data set of 175 protein tertiary structures in 34 different families.

Describes the latest version of these authors' lattice Monte Carlo folding algorithm based on a hierarchy of models of increasing detail. The potential of mean force, predominantly of statistical origin, contains several novel terms that facilitate the cooperative assembly of secondary structure elements and the cooperative packing of the side chains.


A comprehensive review of techniques for optimization of energy functions.


A short summary of one approach to ab initio protein structure prediction.


Folding of four-helix bundles is simulated using a genetic algorithm.


An evaluation of three homology models submitted to the Asilomar conference on 'The Critical Assessment of Techniques for Protein Structure Prediction'. The models span a range of difficulty, from easy (human nucleoside diphosphate kinase), through medium (mouse cellular retinoic acid and binding protein 2), to difficult (human eosiophil neutrotosin).


The relationships between proteins in sequence and structure databases are described. The number of different families is estimated and the growth in sequence and structure data is analyzed.


Computational complexity of the threading problem is investigated.


Describes a new template matching method that does not rely on a scoring function determined by the use of a database of known structures. Instead, direct energy calculations are performed.


Structure comparison methods are reviewed.


67. Henikoff S, Henikoff JG: Protein family classification based on searching a database of blocks. Genomics 1994, 19:97-107. The BLOCKS template searches are available as an Internet service at the WWW address http://www.blocks.fhcrc.org. This service can be used to find sequences related to a given sequence.


72. Describes an improvement of the original three-dimensional-profile method that involves an expression of residue preferences as a continuous function of environmental variables. This leads to a more sensitive method for detecting remote sequence-structure relationships.


A new method to build a complete protein structure from Cα coordinates is presented. An approximate backbone is first generated using geometrical criteria only. Then, the backbone is refined and side chains are placed through the use of exhaustive molecular dynamics simulation. The method is used to generate full-atom models of two proteins from their low-resolution Cα traces.


SCAN3D, a new database system for integrated sequence-structure analysis is introduced. Site-dependent side-chain rotamer distributions can be easily obtained by extracting short segments with a given main-chain conformation. These rotamer distributions can then be used in side-chain modelling.


The authors improve an earlier method by relaxating distance constraints to the target sequence that are transferred from the template structure. This facilitates three-dimensional embedding and energy minimization, and increases the rms between the template and the model; however, it does not appear to increase the accuracy of the models relative to the template structure.


An update on MODELLER that describes the use of the CHARMM force field (see [112]) to restrain the stereochemistry of the comparative models.


An update on MODELLER that describes the use of the CHARMM force field (see [112]) to restrain the stereochemistry of the comparative models.


Database and conformational search methods for loop modelling are compared. It is demonstrated that little correlation exists between the similarity in the anchor and loop regions of two segments and that the database of segments is sparse for segments longer than eight residues. The systematic search procedure can generate almost all structures of short segments in proteins and is thus the preferred method for modeling loops.


An original method for loop modeling by the same authors is combined with multiple copy sampling to increase the accuracy of its efficiency by up to a factor of five. It is also shown that the variability in the predicted loop conformations can be used to estimate the accuracy of the methods.


A method for modeling loops on a given framework is described. Possible loop conformations are generated by randomly combining dimensions from a database of dimers. The predicted loop is the conformation that maximally satisfies the loop closure condition and does not have any atom--atom overlaps. Additional filters, such as disulfide bonds, can be easily imposed on the construction of loops.


The bond-scaling-relaxation algorithm by Zheng et al. [144,145] is used to model more than one loop at the same time. More accurate predictions are invariably obtained.


This method is based on a rotamer library and refines iteratively a conformational matrix of the side chain of a protein such that its current element i,j, at each cycle, gives the probability that the corresponding side-chain i adopts the conformation of its possible rotamer j. Each residue is influenced by the average of all possible environments, weighted by their respective probabilities. The final prediction corresponds to the rotamer with the highest probabilities. Estimates of the conformational entropy of side chain in the folded proteins are also given.


The method described uses a database scheme to generate possible fragments for modeling gaps, and a rotamer library to define possible side-chain conformations. It then iteratively refines the probabilities that the backbone corresponds to database fragment j and side chain corresponds to rotamer for each residue, which experiences the average of all possible environments. The energy function includes only van der Waals terms.


3899 β-turns from 205 protein structures are used to derive β-turn position potentials. Many positional preferences can be rationalized in terms of hydrogen bonds. Preferences for amino acids to adopt a particular conformation in φ, ψ space, and the involvement of some turns in β-hairpins.


The conformational properties of tight two-residue β-turns are examined by empirical energy function calculations. It is shown that the conformation of such turns is determined by the twist of the β-sheet and a local electrostatic effect.


Describes a general method for calculating protein three-dimensional structures using a detailed energy function and external constraints. The method can be used for comparative modelling when atomic positions are restrained to those in template structures, loop modelling when regions delimited short loops are defined, and side-chain modelling when the backbone is kept fixed.


The method described involves the comparison of the local environment of each residue whose side chain is to be predicted with a database of local environments for the same residue type constructed from an analysis of high-resolution protein structures. Local environments are described in terms of the residue type and location of residues that interact with the given side chain. The best few matches are inputted into a Monte Carlo procedure, which gives the final model by removing the steric clashes in the structure.


A Šali, Box 270, The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399, USA.
E-mail: sali@rockvax.rockefeller.edu