JOURNAL OF THE ROYAL SOCIETY Interface

rsif.royalsocietypublishing.org

From local structure to a global framework: recognition of protein folds

Agnel Praveen Joseph[1,2,3,4,†] and Alexandre G. de Brevern[3,4,5,6,†]

[1]Science and Technology Facilities Council, Rutherford Appleton Laboratory, Harwell Oxford, Didcot OX11 0QX, UK
[2]National Centre for Biological Sciences, GKVK, Bellary Road, Bangalore 560065, India
[3]INSERM, U1134, DSIMB, 75739 Paris, France
[4]Université Paris Diderot, Sorbonne Paris Cité, UMR_S 1134, 75739 Paris, France
[5]Institut National de la Transfusion Sanguine (INTS), 75739 Paris, France
[6]Laboratoire d'Excellence, GR-Ex, 75739 Paris, France

Review

Cite this article: Joseph AP, de Brevern AG. 2014 From local structure to a global framework: recognition of protein folds. *J. R. Soc. Interface* **11**: 20131147. http://dx.doi.org/10.1098/rsif.2013.1147

Received: 8 December 2013
Accepted: 25 March 2014

Subject Areas:
computational biology, bioinformatics

Keywords:
bioinformatics, protein fold recognition, local structure prediction, structural alphabets, threading, sequence annotation

Author for correspondence:
Agnel Praveen Joseph
e-mail: agnel-praveen.joseph@stfc.ac.uk

Protein folding has been a major area of research for many years. Nonetheless, the mechanisms leading to the formation of an active biological fold are still not fully apprehended. The huge amount of available sequence and structural information provides hints to identify the putative fold for a given sequence. Indeed, protein structures prefer a limited number of local backbone conformations, some being characterized by preferences for certain amino acids. These preferences largely depend on the local structural environment. The prediction of local backbone conformations has become an important factor to correctly identifying the global protein fold. Here, we review the developments in the field of local structure prediction and especially their implication in protein fold recognition.

1. Introduction

A detailed understanding of the function of a protein can be achieved by studying its three-dimensional structure. Insights into the molecular details of protein interactions and enzyme activities are obtained from the three-dimensional structure and the impact of protein structure-based drug design in pharmaceutical development has been impressive [1–3]. Although significant advances have been made in the field of experimental structure determination [4,5], the difficulty and cost associated with this limit the rate at which protein structures are solved. The sequence information, on the other hand, has grown immensely with the help of high-throughput sequencing techniques [6–11]. The huge gap between the sequence and structure space is formed by sequences whose three-dimensional structures are unknown.

Computational approaches provide effective ways for filling this gap. The methods used for structural annotation assign a probable three-dimensional fold for a given sequence. Applications of computational structure modelling have been enormous [12]. The modelled structures are useful in studying protein–protein [13] and protein–ligand interactions [12–14]. They are also used as search models in experimental structure determination [15] and for characterizing structures of huge assemblies [16–18]. Computational model-based drug design [19,20] and studies for understanding protein dynamics based on modelled structures [12,21,22] have been successful. Large-scale proteome-wide structural annotation studies have also been designed upon computational approaches for fold identification [23,24].

Modelling protein structures based on a template fold (comparative/homology modelling) is so far the most reliable technique for generating three-dimensional structure for a given sequence [25]. The most critical step in comparative modelling is the identification of the correct template fold. The possible number of protein folds are estimated to be limited in number and the currently available structures can cover most of it [26,27]. The ability to relate sequences to the correct folds forms the basis for protein fold recognition. The simplest means for deriving structural information is by direct comparison with other related sequences with known structures [28–30] (figure 1*a*). The tertiary

[†]These authors contributed equally to this study.

Royal Society Publishing

© 2014 The Author(s) Published by the Royal Society. All rights reserved.

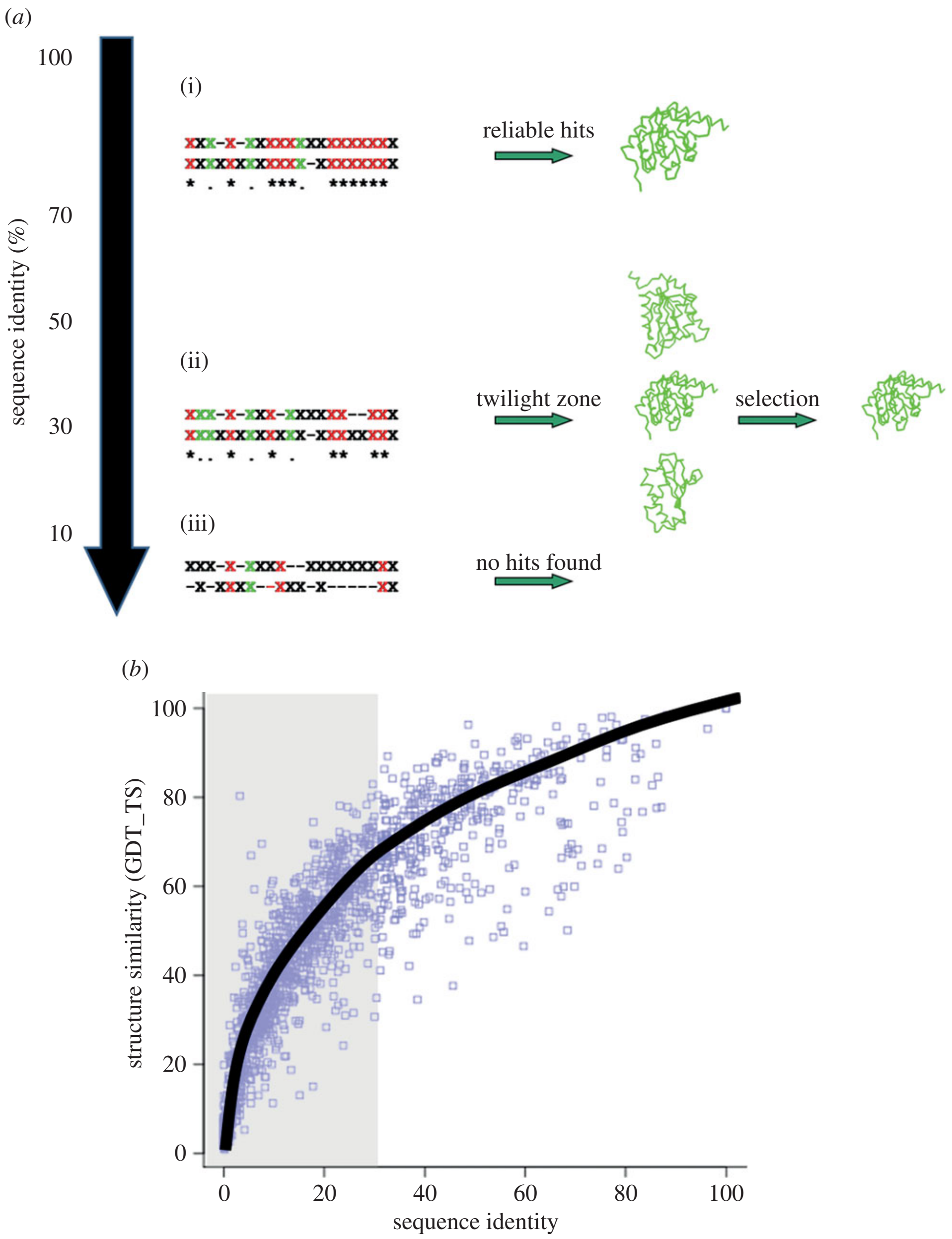


Figure 1. 'Homology' detection with variation in sequence identity. (*a*) Schematic demonstrating the use of sequence comparison for detecting structural homology. The sequence alignments are indicated with 'X' representing any amino acid. Same amino acids at equivalent positions are highlighted in red, similar ones are in green. At sequence identity levels above 30% (i), simple sequence alignments are largely sufficient to detect similar folds. Below this similarity threshold, the alignments are less accurate and thus less efficient in detecting genuine relationships. (ii) Between 20 and 30%, the correct fold is not often detected as the top hit. (iii) At very low sequence identities, simple sequence alignments are not very useful. (*b*) Variation of structural similarity (quantified in terms of GDT_TS score) [31] with change in sequence identity. Even at low sequence identities (less than 30%, highlighted in grey background) significant structural similarity could be observed. (Online version in colour.)

structure of a protein is highly conserved across different species, when compared with its sequence [32]. Figure 1*b* shows the direct relationship existing between structural similarity in terms of global distance test (GDT) [31] and sequence identity. This analysis has been performed on a randomly chosen subset of about 500 SCOP domain, which were structurally aligned [33,34] with iPBA superimposition method [35,36]. Thus, if the sequence similarity (usually quantified as the percentage amino acid identity) is high, the structures are likely to be similar. Comparative modelling can generate reliable structural models (1–3 Å root mean squared deviation (RMSD) with the real structure), especially when the sequence identity is above 30% [25,37] (figure 1*a*(i)).

Nonetheless, the sequences of homologous protein structures can diverge beyond the point where pairwise sequence comparison methods fail. Figure 1*a* highlights the success in detecting the correct fold declines with decrease in sequence identity. The match between the sequence of interest (target) and the known folds is obtained as an alignment of sequences. Below 30% sequence identity (*twilight* zone, figure 1*a*(ii)), the accuracy of alignments falls significantly which results in a low sensitivity in fold recognition. At very low sequence identities (below 15%, figure 1*a*(iii)), the sequence alignments are not very informative. The values provided in the figure are just indicative of the sequence identity ranges, and no fixed thresholds have been characterized [38]. The lectin superfamily is a classic example of this case where the sequence identities fall below 10% but they are known to adopt a jelly-roll-sugar binding fold [39], and a random alignment is expected to have about 12% identity.

In this review, we focus mainly on the scenario where the target has only remote homology with a protein of known fold (template). In such cases, a clear understanding of the association of amino acid sequences with the three-dimensional structure (sequence–structure relationship) is the key factor in determining the success to recognize correct folds. Firstly, a general outline of the methods for fold recognition based on the detection of remote homology is presented. Then the role of local structure prediction in fold recognition is emphasized, followed by a discussion on the importance of careful extraction of sequence–structure relationships.

2. Fold recognition by remote similarity detection

In the absence of closely related proteins of known structure, it is difficult to assign the correct fold using simple sequence alignments (figure 1*a*). Additional information is required to enhance the sensitivity to detect more distant relationships. The knowledge on the evolutionary and structural constraints associated with the target sequence forms the major contributing factor in detecting distant relationships.

2.1. Evolutionary information: profile–profile alignments

The protein sequence databases nowadays contain homologous sequences from different species. A multiple sequence alignment (MSA) can be used to trace the extent of evolutionary divergence among the related sequences. As against a single sequence, MSA consists of information about amino acid propensities at each position in the sequence which characterizes a sequence 'profile'. This information is highly valuable in differentiating conserved and variable positions, and these position-specific amino acid propensities are more effective in identifying a protein that is evolutionarily related to this group. Thus, the use of MSAs as sequence profiles has a huge impact on homology detection (figure 2*a*,*b*) [40,41].

A sequence profile is often described as a position-specific scoring matrix (PSSM), which holds information on amino acid propensities for each position in the sequence, derived based on an MSA. PSI-BLAST and RPS-BLAST [42,43] are widely used sensitive PSSM-based search algorithms for remote homology detection. The search for related sequences is usually carried out iteratively where the profile (PSSM) gets updated at each step with new sequence information. These new sequences, which can be termed as 'intermediates', could also be used as new targets to trace the linkage to the distant homologue [44,45]. Recently, a method named HangOut has been proposed to improve the sensitivity of PSI-BLAST searches especially for domains with long insertions [46].

Several approaches have been proposed to improve the search for related proteins based on sequence profiles. An MSA can be used to generate hidden Markov models (HMMs), which can also be used as efficient search tools [47–50]. HMM-based profile comparison tools are reported to have a higher accuracy than PSSM-based methods [45,51]. To improve the accuracy of profiles generated, efficient methods that can weigh conserved sequence regions have also been developed [52–56]. Amino acid substitution probabilities that drive the alignment scores are influenced by the local structural [57] or sequence neighbourhood [58,59] in the protein. CSI-BLAST [60] uses mutation probabilities from context-specific profiles based on 13-residue windows while DELTA-BLAST [61] incorporates frequencies from longer profiles corresponding to conserved domain sequences [62]. Two iterations of CSI-BLAST [60] are more sensitive than five iterations of PSI-BLAST, whereas DELTA-BLAST [61] is more sensitive than both CS-BLAST and PSI-BLAST in detecting relatively similar and remote homologues. Profile–profile comparison methods that optimize local alignments are also reported to be more effective in remote homology detection [55,63,64].

Fast and efficient HMM profile-based iterative search approaches similar to PSI-BLAST marked further advancements in this field. JACKHMMER [65] relies on a series of database filtering steps to reduce search time, whereas HHBLITS implements HMM–HMM comparisons with approximation of profile columns using 219 extended alphabets [66]. The sensitivity of HHBLITS in detecting remote homologues was about 50–100% more than that of PSI-BLAST.

Increasing amount of evidence of sequence permutations, duplications or fusion events [67–69] raises the need for developing methods that can detect homologues independent of the sequence order. A classic example is the methyl transferase family where gene fusion and permutation events are observed in the evolutionary process resulting in differences in substrate recognition and catalytic activity [70,71]. Interesting developments have also been made in this direction. A few alignment-free techniques were developed for this purpose [72,73], while the majority of methods that employ sequence-order-independent comparisons are optimized for multi-domain proteins [74–77].

However, at a sequence identity well below 20%, the chances of detecting true templates even with profile-based approaches remain low [78]. It is possible that the fold which the sequence adopts is already known but the sequence-based searches fail to trace such relationship. Such cases could be addressed if one can verify whether this protein sequence is compatible with any of the available protein folds. The question is how to derive this compatibility with known folds, given a sequence target.

2.2. Structure information: fold recognition by threading

Two proteins sharing the same fold could have diverged significantly in terms of sequence and it is often difficult to detect the relationship based on sequence- or profile-based methods. Some well-known examples of these are the oligonucleotide/oligosaccharide binding fold, cupins, TIM barrels, serine proteases, etc. Hence it becomes necessary to relate target sequence with the known folds. To assess the fit of a sequence on a template protein fold, it is necessary to quantify the preference for amino acids to occur in the structural environment of the template. This measure can enable us to generate an alignment between the sequence and the template structure (*threading*). Two major strategies have been used to score the sequence–structure compatibility.

2.2.1. Threading based on global potentials

The more direct approach would be to fit each residue from the query sequence onto each position in the template backbone and check whether it is energetically compatible

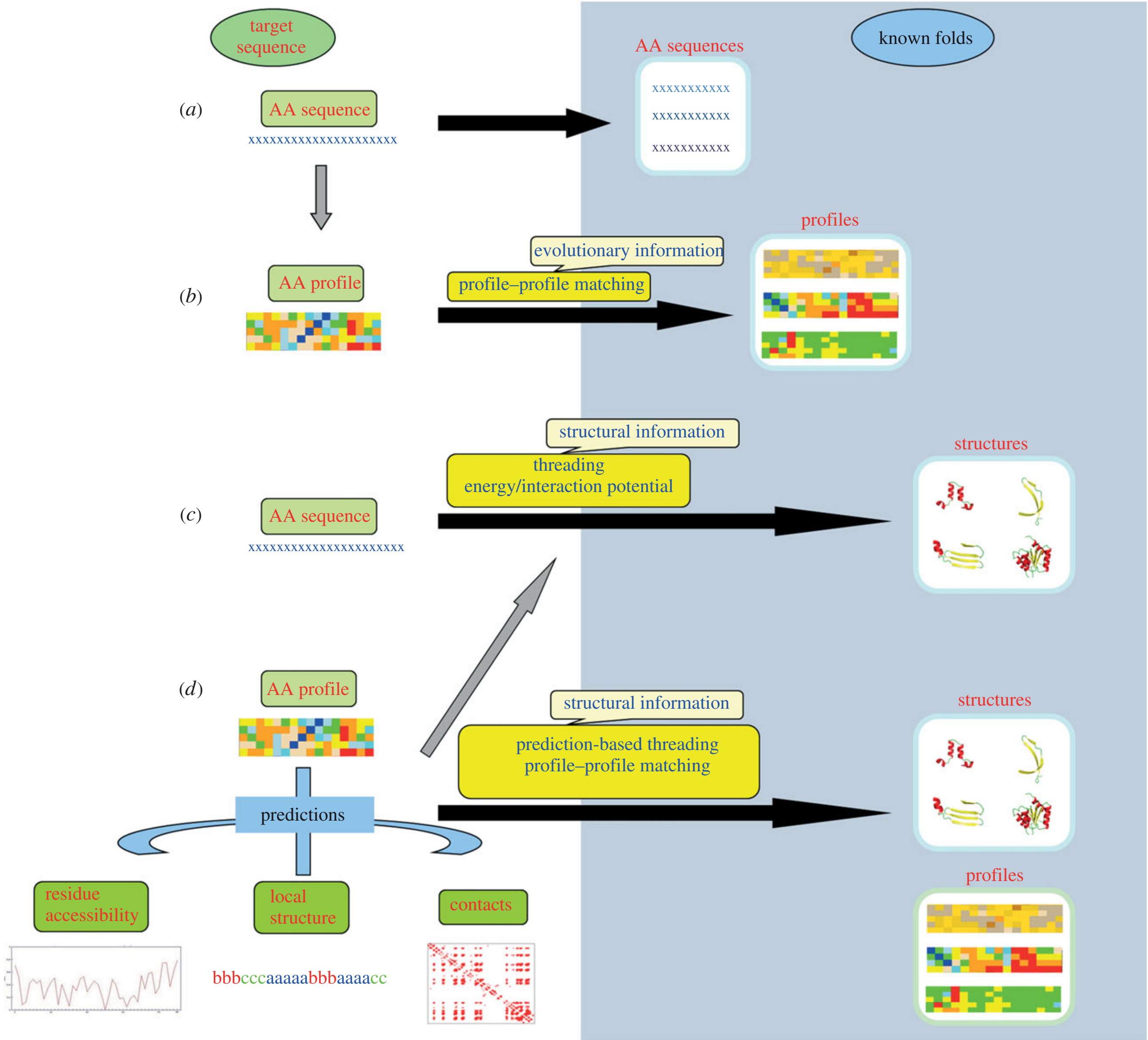


Figure 2. Different strategies for protein fold recognition. The fold space is highlighted by the blue background and the lengths of the black arrows joining the target sequence (space) and fold space give an idea of the distance of relationship. (*a*) Close relationships are often detected by simple sequence alignment techniques. (*b*) Addition of evolutionary information using sequence profiles derived from MSAs helps in detecting more distantly related folds. When the sequence-based alignments are not informative, sequence–structure matching needs to be carried out. (*c*) The target sequence can be threaded on to the known folds to check the compatibility. The compatibility is usually quantified based on the global interaction or energy potential. Obtaining an optimal alignment between the sequence and a fold is however difficult and computationally quite expensive. (*d*) The other alternative is to carry out prediction of different structural features like local backbone conformation, solvent accessibility or contact order and then matching the predicted features with that found in the known fold. (Online version in colour.)

(figure 2*c*). The energy could be quantified as pairwise interaction potentials [79,80] or solvation energy [81] which were used by many of the earlier methods for fold identification [82–84]. The non-local regions of the target residue could have significant difference when compared with that of the template. This means that the equivalent regions (or residues) have to be known *a priori* to calculate the compatibility score. Hence the target–template alignment has to be generated in advance and this makes the problem extremely hard.

An easier solution is the ‘frozen approximation’ [81], where each residue in the target is scored against the structural environment formed by the neighbouring template residues. However, as mentioned earlier, the target and the template often have different environments involving variation in the amino acid residues. Hence this method is frequently prone to accumulate errors that affect the final estimation of compatibility. Approximate solutions to identify residue neighbours could be derived using Monte Carlo simulations [85] or double-dynamic programming [83]. The divide-and-conquer approach used in Prospect [86] aims to solve several sub-structure alignments and integrate them in an optimal way. The complexity associated with fold identification purely based on global interaction potentials was alleviated by combining local structural information [87] and the local structural context is observed to have the major role in obtaining an optimal template alignment [88].

2.2.2. Threading based on local scoring functions

The second category of methods adopts a more localized approach for quantifying the compatibility of the target sequence on a template. They score the preference for each residue to occur in a local environment of the template structure. The preference for an amino acid to occur in a local structural environment can be referred to as the sequence–structure relationship. As mentioned earlier, several structural features can be used to describe the local structural environment of a protein. The most commonly used features are secondary structure, solvent accessibility, residue depth, backbone conformation, potential for hydrogen bonding or hydrophobic interactions [57,89]. A combination of some or all of these features can define one local environment. The issue is to choose the right set of features and weigh each feature to derive the combination.

Two different ways in which the preference of an amino acid for a local structural environment can be calculated are: (i) the amino acid preferences associated with a local structural environment can be extracted as a one- to three-dimensional substitution matrix which gives the score for assigning each amino acid in this environment [57,90–92]. These methods are based on the premise that the amino acid substitution patterns vary based on their structural environments [57]. (ii) The other strategy adopted by most of the recent methods is to predict the structural features separately based on the target sequence and then combine the predictions carefully to search for related structures (figure 2*d*) [93]. The use of predicted local structural features as strings can bring down the problem of sequence–structure comparison to that of a sequence–sequence comparison. As in the case of classical sequence alignment procedures, dynamic programming algorithm can be used to achieve this task [94]. Recognition of protein folds based on predicted local structural features is competitive with methods that consider non-local structural details [95–98].

The efficiency of different profile comparison methods is coupled with the predicted structural information to improve the quality of profile-based fold recognition [54,95,99–106]. The successful approaches for fold recognition use profile comparisons to obtain a limited set of alignments which are then evaluated and refined using the energy-based potentials [101,102,107,108]. An interesting recent observation is that the accuracy of fold recognition improves with the use of artificially evolved sequences compatible with the template folds [109]. These sequences are optimized to stabilize a given structure by simulated annealing based on a variety of potentials, similar to those commonly used in protein threading.

In the following sections, we discuss the developments in the field of local structure prediction which has been the most essential component of prediction-based threading methods.

2.2.2.1. Secondary structure prediction

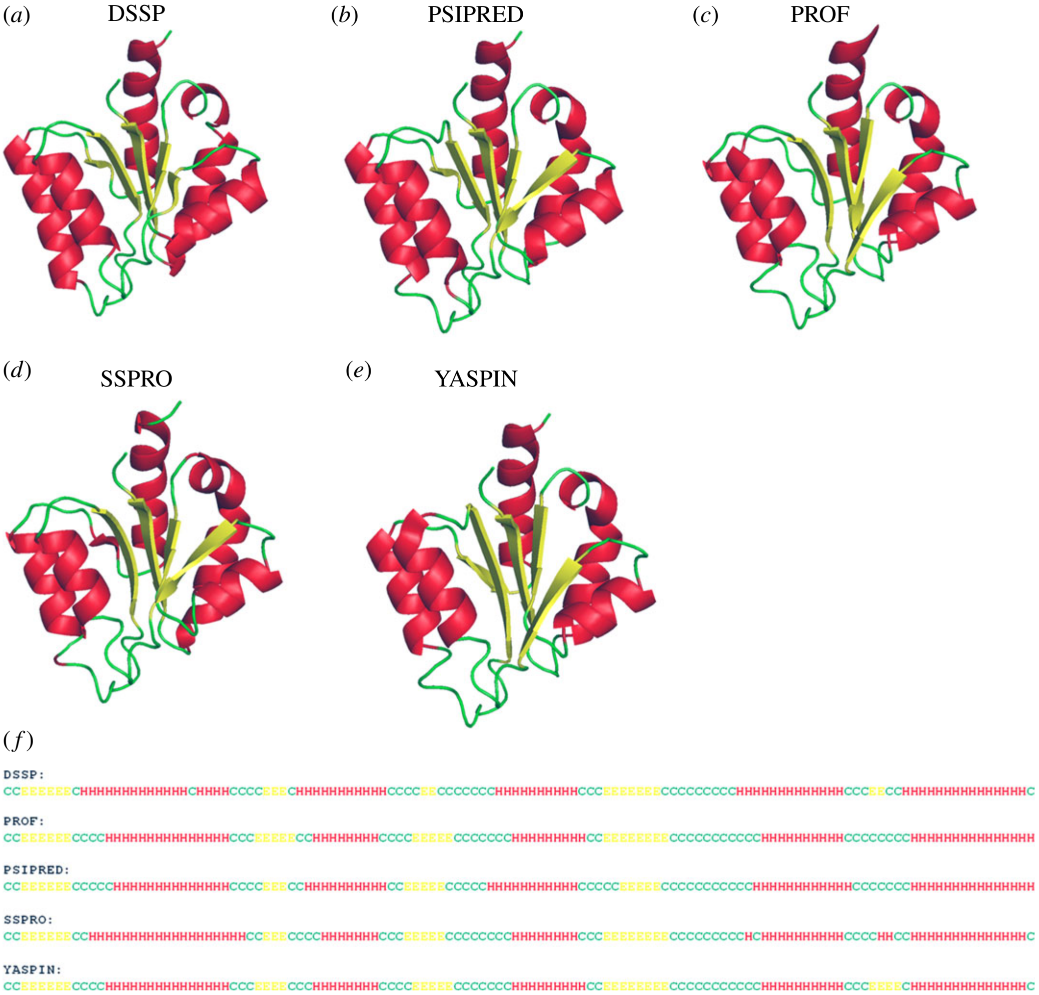
The protein backbone prefers mainly a limited number of stable conformations constrained by the backbone dihedral angles and hydrogen bonds. The two important regularities seen in the local conformation of the backbone constitute α-helices and β-strands [110]. The rest of the backbone is usually considered as coils which represent that not assigned to one of these two conformations. These three states (helix, strand and coil) constitute the classical definition of secondary structures. This description has been used either to complement sequence-based searches or to identify the best structural relative from the results of sequence-based searches. It has been a strategic step in most of the fold recognition approaches [89,95,108, 111,112]. The fold recognition methodologies like PHYRE [95], MUSTER [113], SPARKS [114] and SP5 [105] incorporate methods for predicting secondary structure of the target sequence and then comparing that with the secondary structure assigned for the templates.

Efficient machine learning methods like artificial neural networks (ANNs) [115] or support vector machines (SVMs) [116] are being developed to predict secondary structure from a protein sequence. They are trained to learn the amino acid preferences associated with different secondary structures and make predictions for new sequences. Most of the successful and recent approaches use the information on amino acid propensities derived from sequence profiles [115,117–122]. The use of multiple sequences (as a profile) adds details on amino acid variability at each position in the sequence. Studying the association of relatively large fragments of about 15–20 residues with a secondary structural state helped to incorporate the effect of long-range interactions to a certain extent [123].

It must be noted that 'the machine learning algorithms predict what they learn'. The secondary structure assignments for known structures are made using a non-redundant dataset of protein structures and then the sequence association with each secondary structural unit is learnt. There are several methods available for assigning secondary structures, based on local backbone dihedral angles or hydrogen bonds. It is interesting to note that the assignments made by different methods do not agree to a significant extent [124–126]. Figure 3 presents the comparison of secondary structure assigned based on the three-dimensional structure of methyglyoxal synthase [127] with that of predictions made by different methods. The ambiguities are generally seen in predicting the short repetitive structures and especially boundaries of secondary structures. The underlying cause is that the capping regions do not necessarily follow the same rules set for defining the backbone conformation of helices and strands [132].

Taking into account the variation of secondary structure assignments among different conformations of the same structure (inherent flexibility) and between close structural homologues, a theoretical limit of 88% was proposed for secondary structure prediction accuracy [123]. The most popular and not very recent methods like PSIPRED [115], PROF [122,123] and SSPRO [130] could already achieve a prediction accuracy close to 80%. PHD [129] and PSIPRED [115] use two levels of neural network predictions where the initial predictions are refined at the second step. SSPRO [130] implements a recursive single neural network instead of multiple layers. The use of dual layer SVMs also gave performance comparable to these methods [116,119]. A few improvements or alternative methods have been proposed in the last few years [116–118,120,121,131,133–136] but the increase in prediction accuracy is minimal. Use of consensus approaches based on the results of multiple predictions [133,136] gave accuracy above 80%. The use of a dictionary of words of amino acid stretches associated with a secondary structure state also resulted in predictions comparable to the best machine learning methods available [137].

A recent assessment of available secondary structure prediction methods suggests that a prediction accuracy of about 82% could be reached [138]. The exposed coils were predicted higher than helices and strands. The accuracies of prediction for residues in 3_{10} helices and β-bridges were less than 50% and 48%, respectively [138]. Complementing profile–profile comparisons with predicted secondary structure resulted in a significant improvement in the efficiency of fold recognition [95,108,139,140]. The prediction of



```
DSSP:
CCEEEEEECHHHHHHHHHHHHHCHHHHCCCCEEECHHHHHHHHHHHCCCCEECCCCCCCHHHHHHHHHHCCCEEEEEEECCCCCCCCCHHHHHHHHHHHHHCCCEECCHHHHHHHHHHHHHHHC

PROF:
CCEEEEEECCCCHHHHHHHHHHHHHHHCCCEEEEECCHHHHHHHHCCCCEEEEECCCCCCCHHHHHHHHHCCEEEEEEEECCCCCCCCCCCHHHHHHHHHHCCCCCCCCHHHHHHHHHHHHHHH

PSIPRED:
CCEEEEEECCCCCHHHHHHHHHHHHHHCCCCEEECCHHHHHHHHHHCCEEEEECCCCCHHHHHHHHHHHCCCCCEEEEECCCCCCCCCCCHHHHHHHHHHHHCCCCCCCHHHHHHHHHHHHHHH

SSPRO:
CCEEEEEECCHHHHHHHHHHHHHHHHHHHCCEEECCCCHHHHHHHCCCEEEEECCCCCCCCHHHHHHHHCCCEEEEEEEECCCCCCCCCHCHHHHHHHHHHCCCCHHCCHHHHHHHHHHHHHHC

YASPIN:
CCEEEEEECCCCHHHHHHHHHHHHHHHCCCEEEECCCHHHHHHHHCCCCEEEEECCCCCCCHHHHHHHHHCCEEEEEEEECCCCCCCCCCCHHHHHHHHHHCCCEEEECHHHHHHHHHHHHHHC
```

Figure 3. Comparison of secondary structure prediction methods. For a recently solved structure of methylglyoxal synthase [PDB ID 2X8W] [127], the assigned secondary structure by (*a*) DSSP [128] and predicted ones are shown. The α-helices are shown in red, β-strands in yellow and coils in green. Different secondary structure prediction methods are shown: (*b*) PSIPRED [115], (*c*) PROF [129], (*d*) SSPRO [130] and (*e*) YASPIN [131]. The predictions are also shown as sequence alignment in (*f*). Helices, strands and coils are indicated by H, E and C, respectively. (Online version in colour.)

non-local structural details mainly based on the predicted local features has also reported some success. The prediction of protein contact order which reflects the average length of sequence separating contacting residues, with the aid of predicted secondary structure information is reported to achieve a correlation of about 0.85 [141].

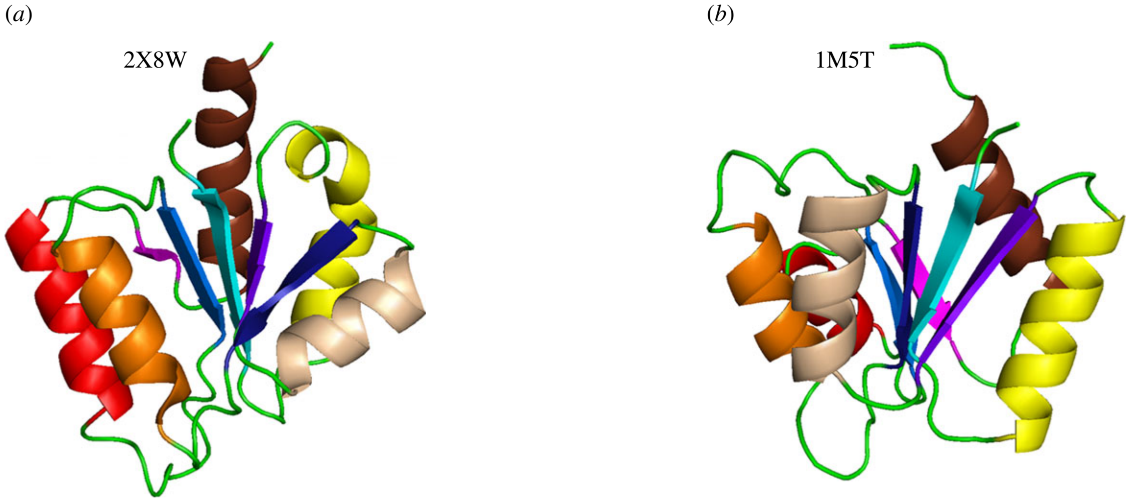
2.2.2.2. Beyond secondary structures

In the absence of reasonable sequence similarity, the secondary structure prediction makes a huge contribution to remote homology detection. However, proteins with similar secondary structure topology need not necessarily have the same tertiary fold. Figure 4 gives an example of one such case where a wrong fold is chosen purely based on the similarity in secondary structure. This is often true for proteins with repeating secondary structural elements, i.e. helix or strand repeats. The three-state secondary structural information (helix, sheet and coil) does not give precise details of the backbone conformation for the complete protein backbone [111]. More than 50% of the residues in the available protein structures are assigned to the coil state which by definition corresponds to irregular backbone conformations. The loss of information caused by considering such a large majority of residues as coils is a main contributor to the recognition of wrong folds [112,144].

On the contrary, the coil state is not strictly irregular. Next to helices and strands, turns characterize another frequent regularity in the protein backbone [145]. β-Turns account for about 25% of the residues in proteins [146]. Other favourable local structures involve PolyProline II helices [147], hairpin loops, corner motifs, β-bulges, etc. [148]. Most of these repeating units are associated with strong sequence–structure relationships. Thus, a major portion of protein backbone can be associated with repeating local structural elements.

Attempts have been made to generate a minimum set of local backbone conformations, a combination of which can be used to represent most or all of the protein backbone conformation. A set of prototypes of local structures that can be used to approximately define the conformation of a protein backbone is called as a structural alphabet (SA) [149]. The number and size of these fragments vary depending on the approach used. Apart from their use in modelling, structural alignment and functional analysis, these libraries also help to extract precise information on the amino acid preferences associated with local structures.

Several approaches have been developed for the prediction of local structures based on fragment libraries [150–157]. The underlying methods used for prediction are based on both probabilistic [150,153,158] and machine learning algorithms [152,159–162]. Bystroff and Baker generated a



(c)

```
>2X8W
CCEEEEEECCCCHHHHHHHHHHHHHHHHCC_EEEEECCHHHHHHHHH__CCCEEEEECCCCC__HHHHHHHHHHCCCCC____EEEEEECCCCCCCC
CCCHHHHHHHHHHCCCCEE__CC__HHHHHHHHHHHHHHHC
>1M5T
CCEEEEE_CCC_HHHHHHHHHHHHH___CCCEEEEECCHHHHHHHHHHHCCCEEEEECCCCCCCHHHHHHHHH_CCCCCCCCCEEEEE_CCCC____
___HHHHHHHHH_CCCCEEEECCCCHHHHHHHHHHHHH__C
```

(d)

```
>2X8W
zzcdddddfklmmmmmmmmmmmmmmmmmpc_cdddfklmmmmmmmno__pacdddddfklop__mmmmmmmmmnopafb____dcddddfkbccfkl
mmmmmmmmmmmmnopacdf__bf__klmmmmmmmmmmmmzz
>1M5T
zzddddd_fbl_klmmmmmmmmmmn___opacdddfbfklmmmmmmmnopabdcddfkbfkbcfklmmmmmmm_pmklmmpccddddf_blcf____
___klmmmmmmn_opabdcdddddfklmmmmmmmmmmp__c
```

Figure 4. Going beyond three-state secondary structure. The structures related to methylglyoxal synthase (*a*) [PDB ID 2X8W] are identified purely based on the secondary structure, using SSEA server [142]. A different fold (*b*) (Response Regulator, PDB ID 1M5T) [143] was obtained as a top hit based on the secondary structure content. The secondary structure alignment (*c*) shows that the structures are close based on the secondary structure; however, the folds are different. The equivalent helices and strands are highlighted in the same colour in the two structures (*a*,*b*). A more precise description of the backbone conformation was obtained using PB (*d*). The assignment of PB instead of the three-state secondary structures highlights many differences between the two structures, which were otherwise masked by the secondary structure definition. The segments assigned as coils (indicated by 'C') are highlighted in red in the PB alignment. Other differences in the regular secondary structures are in blue. (Online version in colour.)

library by clustering frequently observed short sequence patterns (I-sites) in protein structures. An efficient local structure prediction method was developed based on this library [150,163]. As the number of prototypes increase, the prediction accuracy was found to be lower. However, the regions predicted with high confidence add fine details on structural motifs and local backbone conformation. The use of secondary structure information is proved to improve the accuracy of local structure prediction. However, the repetitive structures are sometimes over-predicted in place of other local conformations [164].

Protein blocks (PBs) are a widely used SA composed of 16 pentapeptide conformations characterized by the series of backbone dihedral angles [153]. These conformations are characterized by strong sequence–structure relationships determined by the high predictability. Figure 4*d* gives an example of two different protein folds having the same secondary structure topology. It highlights that the PB description is more informative when compared to the three-state secondary structure, especially for the coil regions. It also underlines the significance of precise local structural information in fold recognition. A series of methods have been tested for the prediction of PBs from sequence data. Bayesian prediction models gave an accuracy ranging from 34 to 48% [153,164–166]. Dual layer ANNs and SVMs improved the prediction rate to about 58.5% [159], 61% [167] and 67% [160]. Combining information on secondary structure and solvent accessibility further enhanced the prediction rate of PBs [168]. Longer fragments based on PBs were predicted with better accuracy [166,169]. Frequently observed 11 residue fragments described based on the dihedrals were predicted with a significant accuracy of about 63% using SVMs [170]. Prediction of structural flexibility based on local structure preferences has also been successful [171]. Table 1 gives a list of popular and more recent methods for local structure prediction, based on fragment libraries.

In recent years, a few attempts have been carried out to predict the φ/ψ backbone dihedral angles associated with each residue, based on the preferences observed in the

Table 1. Fragment-based local structure prediction methods. The table gives the list of methods for predicting local backbone conformation based on a library of fragments (prototypes). The length, number of prototypes and the distance measure used to generate the library are also mentioned. The reported prediction rates from the original publication are also listed. MDA, maximum deviation in torsion angles; ANN, artificial neural networks; SVM, support vector machines.

research team	fragment length	distance measure	prediction method	prototype number	prediction rate (%)
Rooman *et al.*	4, 5, 6, 7	Cα RMSD	statistical mechanics (mean force potential)	4	40–47
Bystroff & Baker	3–19	sequence profiles, RMSD, MDA	profile–profile matching	13 (later updated to 16)	50
de Brevern *et al.*	5	dihedral angles	Bayesian	16	34.4
Hunter & Subramaniam	7	hypercosine Cα	Bayesian	28–16336	40
Yang & Wang	9	dihedral angles	sequence profile matching	138604	79
Etchebest *et al.*	5	dihedral angles	Bayesian (simulated annealing)	16 [153]	49
Benros *et al.*	11	Cα RMSD, PB based	hybrid protein model	120	51.2
Sander *et al.*	7	Cα distance	decision trees, SVM, random forest	28	23–36
Dong *et al.*	7	Cα distance	ANN	28	45.6
Dong *et al.*	5	dihedral angles	ANN	16 [153]	58.5
Zimmermann *et al.*	5	dihedral angles	SVM	16 [153]	61
Chen & Johnson	9	Cα distance	SVM	800	72
Bornot *et al.*	11	Cα RMSD, PB based	hybrid protein model	120	63.1
Rangwala *et al.*	5	dihedral angles	SVM	16 [153]	67
Yu *et al.*	7–19	dihedral angles	Bayesian	82	62

Ramachandran map [172]. The highly preferred regions of dihedral angle pairs define the predicted classes. Both ANN- and SVM-based predictions [173,174] have been carried out, with a special focus on the coil regions. Prediction accuracy of above 80% was reported. Another approach predicting the probable dihedral angle associated with the coil residues has been developed [175]. The favoured dihedral region in the Ramachandran plot was divided into bins (30 × 30) and the probability of occurrence in these bins is predicted. Significant improvement could be achieved over random prediction and about 80% accuracy is reported for the prediction of the top 20 populated bins. Prediction of dihedral angles also helped in improving the accuracy of secondary structure prediction [135]. In this approach based on SVMs, an accuracy of prediction of about 54–57% was obtained by clustering the φ/ψ space into seven regions based on the population distribution.

Absolute values of φ and ψ angles are also predicted by several methods mainly based on ANNs [176–178]. Predicted secondary structure and/or solvent accessibility were combined with the sequence or profile information, as inputs for these learning methods. The ANGLOR method [177] was assessed to provide an error of 28° and 46° for the φ and ψ dihedral angles, respectively. These values were reduced to 22° and 36° with the improved Real-SPINE method [179].

Local structure prediction beyond the three-state secondary structures has been shown to improve recognition of remote homologues [180]. Prediction of absolute values of the dihedral angles has been incorporated in many recent fold recognition tools [105,113]. More than 5% increase in efficiency of detection of homologous folds was reported, with the addition of dihedral angle predictions [105]. A recent study also demonstrated that the use of local structure preference information significantly improves the quality of fold recognition [181].

2.2.3. Fragment-based fold recognition

The local regions of strong sequence–structure relationships are extracted as fragments and are assembled to generate the fold. The recognition of related folds by I-TASSER is mainly based on a profile–profile alignment method [98]. The efficiency of the profile search (see §2.1) is enhanced by the addition of secondary structure information. Various strategies involving HMMs [182], PSI-BLAST profiles [42], global [29] and local [30] dynamic programming algorithms are employed in the profile-based search of I-TASSER. The continuous fragments aligned with regions of multiple templates are reassembled into complete models [183]. The unaligned regions without structurally similar segments in the template are built by *ab initio* modelling, i.e. generating structural models based on physico-chemical and mechanical properties, without the use of a template [184].

In the absence of a template of similar fold, the target protein could still have local structural motifs observed in the available protein structures [185]. Though a homologous fold cannot be identified reliably, a probable model could be generated from a collection of such local motifs. This forms the basis of ROSETTA that generates models from fragments compatible with the sequence [186–188].

Hybrid template-based approaches associate fragment conformations for the sequence and detect distant fold similarities based on the fragment similarities. BBSP (building blocks structure predictor) [189] is one such algorithm that gave better accuracy when compared with COMPASS [190], HHpred [51] and PHYRE2 [95] on a dataset of 100 targets. Local backbone conformations from fragments are also used along with coarse-grained force fields to yield an energy optimized final model [191].

The generation of models by the combination of fragments is a remarkable development which can also enhance de novo structure prediction. SAs or local structure libraries can contribute significantly in this aspect and several modelling approaches based on such fragment libraries have been developed [163,192–196]. A combination of fragment assembly and lattice-based folding has been adopted to achieve fast conformational sampling and refine the models generated [108,197,198].

The length of fragments and the fragment insertion length can be chosen separately for different structural class of proteins; the α-helical proteins are modelled better with large fragment lengths and insertion size, whereas β-strand-rich proteins require shorter fragment insertions [199].

2.2.4. Refining local structure prediction

As mentioned earlier, the residue preferences for a local structure are strongly influenced by the structural environment [57,200–204]. Thus, the prediction of local structures could be enhanced with the information of other structural features. As the amino acid preferences for secondary structures differ between buried and solvent-exposed regions of protein structure [205–207], solvent accessibility is sometimes predicted prior to secondary structure prediction [205]. Addition of accessibility information resulted in the improvement of some probabilistic secondary structure prediction methods [208]. It was also reported that the use of residue-specific accessibility cut-offs and multi-state accessibilities improves the prediction of secondary structures. Discarding active site residues with functional constraints from the learning set also resulted in an improvement in the sensitivity of one- to three-dimensional substitution tables with representing amino acid preferences for local structural environments [209].

The stability of a local structural fragment might be determined by interactions not necessarily constrained within the fragment [203,210–212]. Such cases reflect weak sequence–structure relationships. Considering long sequence windows (15–20 residues) for prediction of local structures helps to include the effects of non-local interactions to a large extent [123]. However, the accuracy of local structure prediction is still limited by the inability to include long-range interactions [213,214]. A systematic identification of weak signals of sequence–structure relationships can help in refining the local structure prediction methodologies. Studies on chameleon sequences characterized by locally unstable structures have been carried out to explore such weak relationships [200,215]. Prediction of local regions of flexibility is gaining equal importance [216,217] and the use of flexibility information can aid in refining the available fold recognition protocols by complementing the local conformation predictions. Figure 5 highlights the high confidence predictions of secondary structures, flexible and disordered regions on glutamate mutase [218]. Structural dynamic profiles may also be used as another feature that can be used for fold detection.

2.2.5. In search of specificities

The knowledge of sequence–sequence and sequence–structure relationships is gained on the premise that it is applicable to all proteins. Even though this is often true, some specificities have also been identified among certain sets of proteins.

The amino acid composition varies between different proteomes. Depending on the organism, the variation could be sometimes quite significant such that the standard substitution matrices may fail to find the right homologues. Based on this perspective, substitution matrices adjusted for the background composition are derived [221]. These matrices are reported to be quite efficient in detecting homologues from the species. Species-specific variations are also observed with respect to amino acid propensities for local protein structures [222]. This observation is quite interesting and the specificities could be exploited in improving the accuracy of approaches for local structure prediction. A study conducted in this direction showed promising results with *Plasmodium falciparum*, *Arabidopsis thaliana* and *Saccharomyces cerevisiae* [223]. Considering the compositions corresponding to both query and template improves profile comparisons [224]. The amino acid preferences for local structures also show variations among different structural classes of proteins [225]. These specific preferences could be carefully exploited to enhance the accuracy of local structure prediction. The information on the specific amino acid preferences has been used to develop fold recognition approaches dedicated for specific classes of proteins [226,227].

2.3. Some successful methods

In order to assess the performance of different methods for protein structure prediction, Moult and co-workers introduced a public experiment called critical assessment of techniques for protein structure prediction (CASP). The participants are provided with protein sequences for which the structures have not been released. The I-TASSER server [108] gave the best performance in the recent CASP assessments (CASP 8, 9 and 10) of fold prediction servers [228]. The other servers in the top category were Robetta [186], Quark [229], HHpred [140], pmodeller [230], RAPTOR [87], pro-sp3 TASSER [231] and FALCON [232].

HHpred uses a novel HMM-based profile comparison method with enhanced accuracy by incorporating information on predicted secondary structure [51]. FALCON employs a fragment-HMM approach [232] where both the preferred dihedral angles of each residue and sequence–structure relationships from fragment libraries are integrated to predict structural models for a sequence.

It has become a common strategy to combine different predicted structural features along with amino acid sequences to improve the accuracy of profile-based fold detection [55,106,177,181,233]. Weighted matching of multiple profiles (sequence and structural features) has also been employed to enhance the accuracy of recognizing homologous folds [105,234].

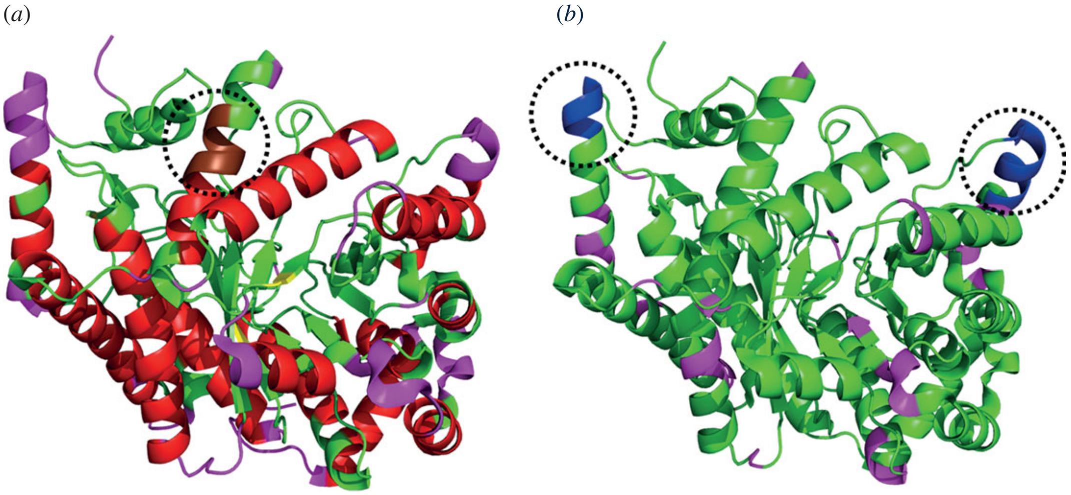


Figure 5. Prediction of local conformational rigidity and flexibility. Structure of glutamate mutase [PDB ID: 1CCW, chain B] [218] (*a*) highlighting the predicted secondary structures [219]: helices (red) and strands (yellow), flexible regions [217] (pink) and a discordant helix with high strand propensity (brown, highlighted within the dotted circle) [215]. (*b*) The flexible regions predicted (probability > 0.5 and confidence > 10) by PredyFlexy method [216] (pink) and the predicted disordered region [220] is shown in blue (highlighted within the dotted circles). (Online version in colour.)

2.3.1. Combining different approaches

One of the main limitations of the available fold recognition procedures is that they often fail to pick the correct template as the top hit. However in most cases, the right fold occurs among the top 10 or 20 hits [235]. The specificity can be improved by assessing the results of several different methods. This idea has led to the development of many meta-servers which run different fold recognition approaches and report the probable folds based on a consensus approach [98,183,236–241]. They also integrate methods for prediction of local structural features and scoring functions to assess and refine the results.

Hybrid methods generate models by combining structures of multiple templates which are either a set of initial predicted models or those obtained from different fold recognition methods [89,198,231,242–245]. Pro-sp3-TASSSER [231] is one such method that identifies template fold based on PROSPECTOR [102] and SP3 [106]. They also involve realignment of target–template in the uncertain regions [197,246,247]. Pcons [230,248] generates structural models based on the target–template alignments generated by different approaches and has been quite competitive in the recent CASP experiments. Nevertheless, a consensus approach may also suppress the true result, if only one of the underlying methods is successful [249].

The recent IntFOLD-TS approach [250] is one of these successful consensus methods; it integrates multiple sequence–structure alignments from methods such as SP3 [106], SPARKS [114], HHsearch [140] and COMA [251] and generates many models with multiple and single templates and scores them. The combination of these methods resulted in a better accuracy in generating models for 117 CASP targets when compared with the component methods individually. eThread [252] is another meta-threading approach that uses a combination of 10 methods and generates consensus alignments using machine learning techniques. This approach is reported to be more sensitive than any of the component methods and nearly 50% of the models were of high quality (TMscore > 0.5) [253].

3. Conclusion

The favourable local backbone conformations and the associated amino acid preferences can be carefully extracted to predict the conformation of a new sequence. Several methods for the efficient prediction of local backbone conformations have been proposed in recent years. It is becoming increasingly clear that these methods can contribute significantly to improve the accuracy of identifying related folds. Careful extraction of sequence–structure relationships is important for reducing the extent of false predictions. The knowledge of preferred local conformations is also used in generating structural models by the assembly of fragments. Strong signals of sequence–structure preferences need to discriminate from weak associations like chameleon sequences, flexible and disordered regions. The use of predicted flexibility profiles of a protein structure may also add to the accuracy of recognizing correct folds. The sequence–structure relationships learnt can be further refined based on specific proteomes or based on the structural class of proteins. Preliminary studies with available data point towards an improvement in prediction accuracy with such dataset-specific learning. Hence an efficient integration of local structural preferences with the global features helps significantly in our efforts for recognizing the fold that an amino acid sequence will adopt.

Acknowledgements. We thank Dr Adam Wilkins (Frontiers in Life Sciences doctorate school (FDV Paris, France), and Biochemistry, Biotherapy, Molecular Biology, Infectiology—B3MI doctorate school (Univ Paris Diderot and Univ Paris Descartes, Paris, France)) for useful discussions and suggestions. The authors also thank Jean-Christophe Gelly, Catherine Etchebest, N. Srinivasan, R. Sowdhamini, R.M. Bhaskara, S. Mahajan and G. Agarwal for fruitful discussions.

Funding statement. This work was supported by grants from the Ministry of Research (France), Univ Paris Diderot (France), Sorbonne Paris Cité (France), the National Institute of Blood Transfusion (INTS, France) and the National Institute for Health and Medical Research (INSERM, France). A.P.J. received a grant from CEFIPRA number 3903-E, and acknowledges NCBS for the post-doctoral fellowship. A.B. acknowledges CEFIPRA for a collaborative grant (no. 3903-E).

References

1. Anderson AC. 2003 The process of structure-based drug design. *Chem. Biol.* **10**, 787–797. (doi:10.1016/j.chembiol.2003.09.002)
2. Chen L, Morrow JK, Tran HT, Phatak SS, Du-Cuny L, Zhang S. 2013 From laptop to benchtop to bedside: structure-based drug design on protein targets. *Curr. Pharm. Des.* **18**, 1217–1239. (doi:10.2174/138161212799436386)
3. Verlinde CL, Hol WG. 1994 Structure-based drug design: progress, results and challenges. *Structure* **2**, 577–587. (doi:10.1016/S0969-2126(00)00060-5)
4. Berman HM *et al*. 2009 The protein structure initiative structural genomics knowledgebase. *Nucleic Acids Res.* **37**, D365–D368. (doi:10.1093/nar/gkn790)
5. Chandonia JM, Brenner SE. 2006 The impact of structural genomics: expectations and outcomes. *Science* **311**, 347–351. (doi:10.1126/science.1121018)
6. Drmanac R *et al*. 2010 Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science* **327**, 78–81. (doi:10.1126/science.1181498)
7. International Human Genome Sequencing Consortium. 2004 Finishing the euchromatic sequence of the human genome. *Nature* **431**, 931–945. (doi:10.1038/nature03001)
8. Lander ES. 2011 Initial impact of the sequencing of the human genome. *Nature* **470**, 187–197. (doi:10.1038/nature09792)
9. Metzker ML. 2010 Sequencing technologies—the next generation. *Nat. Rev. Genet.* **11**, 31–46. (doi:10.1038/nrg2626)
10. Morey M, Fernandez-Marmiesse A, Castineiras D, Fraga JM, Couce ML, Cocho JA. 2013 A glimpse into past, present, and future DNA sequencing. *Mol. Genet. Metab.* **110**, 3–24. (doi:10.1016/j.ymgme.2013.04.024)
11. Parkhill J. 2013 What has high-throughput sequencing ever done for us? *Nat. Rev. Microbiol.* **11**, 664–665. (doi:10.1038/nrmicro3112)
12. Petrey D, Honig B. 2005 Protein structure prediction: inroads to biology. *Mol. Cell* **20**, 811–819. (doi:10.1016/j.molcel.2005.12.005)
13. Zhang QC *et al*. 2012 Structure-based prediction of protein–protein interactions on a genome-wide scale. *Nature* **490**, 556–560. (doi:10.1038/nature11503)
14. Kauffman C, Rangwala H, Karypis G. 2008 Improving homology models for protein-ligand binding sites. *Comput. Syst. Bioinform. Conf.* **7**, 211–222.
15. Bibby J, Keegan RM, Mayans O, Winn MD, Rigden DJ. 2012 AMPLE: a cluster-and-truncate approach to solve the crystal structures of small proteins using rapidly computed ab initio models. *Acta Crystallogr. D Biol. Crystallogr.* **68**, 1622–1631. (doi:10.1107/S0907444912039194)
16. Alber F *et al*. 2007 The molecular architecture of the nuclear pore complex. *Nature* **450**, 695–701. (doi:10.1038/nature06405)
17. Lasker K, Phillips JL, Russel D, Velazquez-Muriel J, Schneidman-Duhovny D, Tjioe E, Webb B, Schlessinger A, Sali A. 2010 Integrative structure modeling of macromolecular assemblies from proteomics data. *Mol. Cell. Proteomics* **9**, 1689–1702. (doi:10.1074/mcp.R110.000067)
18. Topf M, Lasker K, Webb B, Wolfson H, Chiu W, Sali A. 2008 Protein structure fitting and refinement guided by cryo-EM density. *Structure* **16**, 295–307. (doi:10.1016/j.str.2007.11.016)
19. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen MY, Pieper U, Sali A. 2007 Comparative protein structure modeling using MODELLER. *Curr. Protoc. Protein Sci.* Ch. 2, Unit 2.9. (doi:10.1002/0471140864.ps0209s50)
20. Schlessinger A, Geier E, Fan H, Irwin JJ, Shoichet BK, Giacomini KM, Sali A. 2011 Structure-based discovery of prescription drugs that interact with the norepinephrine transporter, NET. *Proc. Natl Acad. Sci. USA* **108**, 15 810–15 815. (doi:10.1073/pnas.1106030108)
21. Shen J, Zhang W, Fang H, Perkins R, Tong W, Hong H. 2013 Homology modeling, molecular docking, and molecular dynamics simulations elucidated alpha-fetoprotein binding modes. *BMC Bioinformatics* **14**(Suppl. 14), S6. (doi:10.1186/1471-2105-14-S14-S6)
22. Tseng CY, Gajewski M, Danani A, Tuszynski JA. In press. Homology and molecular dynamics models of toll-like receptor 7 protein and its dimerization. *Chem. Biol. Drug Des*. (doi:10.1111/cbdd.12278)
23. Drew K *et al*. 2011 the proteome folding project: proteome-scale prediction of structure and function. *Genome Res.* **21**, 1981–1994. (doi:10.1101/gr.121475.111)
24. Muller A, MacCallum RM, Sternberg MJ. 2002 Structural characterization of the human proteome. *Genome Res.* **12**, 1625–1641. (doi:10.1101/gr.221202)
25. Zhang Y. 2008 Progress and challenges in protein structure prediction. *Curr. Opin. Struct. Biol.* **18**, 342–348. (doi:10.1016/j.sbi.2008.02.004)
26. Kihara D, Skolnick J. 2003 The PDB is a covering set of small protein structures. *J. Mol. Biol.* **334**, 793–802. (doi:10.1016/j.jmb.2003.10.027)
27. Zhang Y, Hubner IA, Arakaki AK, Shakhnovich E, Skolnick J. 2006 On the origin and highly likely completeness of single-domain protein structures. *Proc. Natl Acad. Sci. USA* **103**, 2605–2610. (doi:10.1073/pnas.0509379103)
28. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990 Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410. (doi:10.1016/S0022-2836(05)80360-2)
29. Needleman SB, Wunsch CD. 1970 A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J. Mol. Biol.* **48**, 443–453. (doi:10.1016/0022-2836(70)90057-4)
30. Smith TF, Waterman MS. 1981 Identification of common molecular subsequences. *J. Mol. Biol.* **147**, 195–197. (doi:10.1016/0022-2836(81)90087-5)
31. Zemla A. 2003 LGA: a method for finding 3D similarities in protein structures. *Nucleic Acids Res.* **31**, 3370–3374. (doi:10.1093/nar/gkg571)
32. Chothia C, Lesk AM. 1986 The relation between the divergence of sequence and structure in proteins. *EMBO J.* **5**, 823–826.
33. Balaji S, Sujatha S, Kumar SS, Srinivasan N. 2001 PALI—a database of Phylogeny and ALIgnment of homologous protein structures. *Nucleic Acids Res.* **29**, 61–65. (doi:10.1093/nar/29.1.61)
34. Murzin AG, Brenner SE, Hubbard T, Chothia C. 1995 SCOP: a structural classification of proteins database for the investigation of sequences and structures. *J. Mol. Biol.* **247**, 536–540. (doi:10.1016/S0022-2836(05)80134-2)
35. Gelly JC, Joseph AP, Srinivasan N, de Brevern AG. 2011 iPBA: a tool for protein structure comparison using sequence alignment strategies. *Nucleic Acids Res.* **39**, W18–W23. (doi:10.1093/nar/gkr333)
36. Joseph AP, Srinivasan N, de Brevern AG. 2011 Improvement of protein structure comparison using a structural alphabet. *Biochimie* **93**, 1434–1445. (doi:10.1016/j.biochi.2011.04.010)
37. Chakravarty S, Godbole S, Zhang B, Berger S, Sanchez R. 2008 Systematic analysis of the effect of multiple templates on the accuracy of comparative models of protein structure. *BMC Struct. Biol.* **8**, 31. (doi:10.1186/1472-6807-8-31)
38. Rost B. 1999 Twilight zone of protein sequence alignments. *Protein Eng.* **12**, 85–94. (doi:10.1093/protein/12.2.85)
39. Emsley J, White HE, O'Hara BP, Oliva G, Srinivasan N, Tickle IJ, Blundell TL, Pepys MB, Wood SP. 1994 Structure of pentameric human serum amyloid P component. *Nature* **367**, 338–345. (doi:10.1038/367338a0)
40. Ohlson T, Wallner B, Elofsson A. 2004 Profile–profile methods provide improved fold-recognition: a study of different profile–profile alignment methods. *Proteins* **57**, 188–197. (doi:10.1002/prot.20184)
41. Rychlewski L, Jaroszewski L, Li W, Godzik A. 2000 Comparison of sequence profiles. Strategies for structural predictions using sequence information. *Protein Sci.* **9**, 232–241. (doi:10.1110/ps.9.2.232)
42. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402. (doi:10.1093/nar/25.17.3389)
43. Schaffer AA, Aravind L, Madden TL, Shavirin S, Spouge JL, Wolf YI, Koonin EV, Altschul SF. 2001 Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. *Nucleic Acids Res.* **29**, 2994–3005. (doi:10.1093/nar/29.14.2994)
44. Li W, Pio F, Pawlowski K, Godzik A. 2000 Saturated BLAST: an automated multiple intermediate sequence search used to detect distant homology. *Bioinformatics* **16**, 1105–1110. (doi:10.1093/bioinformatics/16.12.1105)

45. Park J, Karplus K, Barrett C, Hughey R, Haussler D, Hubbard T, Chothia C. 1998 Sequence comparisons using multiple sequences detect three times as many remote homologues as pairwise methods. *J. Mol. Biol.* **284**, 1201–1210. (doi:10.1006/jmbi.1998.2221)

46. Kim BH, Cong Q, Grishin NV. 2010 HangOut: generating clean PSI-BLAST profiles for domains with long insertions. *Bioinformatics* **26**, 1564–1565. (doi:10.1093/bioinformatics/btq208)

47. Eddy SR. 1998 Profile hidden Markov models. *Bioinformatics* **14**, 755–763. (doi:10.1093/bioinformatics/14.9.755)

48. Eddy SR. 2009 A new generation of homology search tools based on probabilistic inference. *Genome Inform.* **23**, 205–211.

49. Hughey R, Krogh A. 1996 Hidden Markov models for sequence analysis: extension and analysis of the basic method. *Comput. Appl. Biosci.* **12**, 95–107.

50. Madera M. 2008 Profile Comparer: a program for scoring and aligning profile hidden Markov models. *Bioinformatics* **24**, 2630–2631. (doi:10.1093/bioinformatics/btn504)

51. Soding J. 2005 Protein homology detection by HMM–HMM comparison. *Bioinformatics* **21**, 951–960. (doi:10.1093/bioinformatics/bti125)

52. Morgenstern B, Werner N, Prohaska SJ, Steinkamp R, Schneider I, Subramanian AR, Stadler PF, Weyer-Menkhoff J. 2005 Multiple sequence alignment with user-defined constraints at GOBICS. *Bioinformatics* **21**, 1271–1273. (doi:10.1093/bioinformatics/bti142)

53. Notredame C, Higgins DG, Heringa J. 2000 T-Coffee: a novel method for fast and accurate multiple sequence alignment. *J. Mol. Biol.* **302**, 205–217. (doi:10.1006/jmbi.2000.4042)

54. O'Sullivan O, Suhre K, Abergel C, Higgins DG, Notredame C. 2004 3DCoffee: combining protein sequences and structures within multiple sequence alignments. *J. Mol. Biol.* **340**, 385–395. (doi:10.1016/j.jmb.2004.04.058)

55. Sadreyev RI, Tang M, Kim BH, Grishin NV. 2007 COMPASS server for remote homology inference. *Nucleic Acids Res.* **35**, W653–W658. (doi:10.1093/nar/gkm293)

56. Subramanian AR, Weyer-Menkhoff J, Kaufmann M, Morgenstern B. 2005 DIALIGN-T: an improved algorithm for segment-based multiple sequence alignment. *BMC Bioinformatics* **6**, 66. (doi:10.1186/1471-2105-6-66)

57. Overington J, Johnson MS, Sali A, Blundell TL. 1990 Tertiary structural constraints on protein evolutionary diversity: templates, key residues and structure prediction. *Proc. R. Soc. Lond. B* **241**, 132–145. (doi:10.1098/rspb.1990.0077)

58. Knight RD, Freeland SJ, Landweber LF. 2001 A simple model based on mutation and selection explains trends in codon and amino-acid usage and GC composition within and across genomes. *Genome Biol.* **2**, research 0010. (doi:10.1186/gb-2001-2-4-research0010)

59. Yu YK, Wootton JC, Altschul SF. 2003 The compositional adjustment of amino acid substitution matrices. *Proc. Natl Acad. Sci. USA* **100**, 15 688–15 693. (doi:10.1073/pnas.2533904100)

60. Biegert A, Soding J. 2009 Sequence context-specific profiles for homology searching. *Proc. Natl Acad. Sci. USA* **106**, 3770–3775. (doi:10.1073/pnas.0810767106)

61. Boratyn GM, Schaffer AA, Agarwala R, Altschul SF, Lipman DJ, Madden TL. 2012 Domain enhanced lookup time accelerated BLAST. *Biol. Direct* **7**, 12. (doi:10.1186/1745-6150-7-12)

62. Marchler-Bauer A *et al*. 2011 CDD: a Conserved Domain Database for the functional annotation of proteins. *Nucleic Acids Res.* **39**, D225–D229. (doi:10.1093/nar/gkq1189)

63. Becker E, Cotillard A, Meyer V, Madaoui H, Guerois R. 2007 HMM-Kalign: a tool for generating sub-optimal HMM alignments. *Bioinformatics* **23**, 3095–3097. (doi:10.1093/bioinformatics/btm492)

64. Panchenko AR. 2003 Finding weak similarities between proteins by sequence profile comparison. *Nucleic Acids Res.* **31**, 683–689. (doi:10.1093/nar/gkg154)

65. Johnson LS, Eddy SR, Portugaly E. 2010 Hidden Markov model speed heuristic and iterative HMM search procedure. *BMC Bioinformatics* **11**, 431. (doi:10.1186/1471-2105-11-431)

66. Remmert M, Biegert A, Hauser A, Soding J. 2012 HHblits: lightning-fast iterative protein sequence searching by HMM–HMM alignment. *Nat. Methods* **9**, 173–175. (doi:10.1038/nmeth.1818)

67. Bashton M, Chothia C. 2007 The generation of new protein functions by the combination of domains. *Structure* **15**, 85–99. (doi:10.1016/j.str.2006.11.009)

68. Todd AE, Orengo CA, Thornton JM. 2001 Evolution of function in protein superfamilies, from a structural perspective. *J. Mol. Biol.* **307**, 1113–1143. (doi:10.1006/jmbi.2001.4513)

69. Vesterstrom J, Taylor WR. 2006 Flexible secondary structure based protein structure comparison applied to the detection of circular permutation. *J. Comput. Biol.* **13**, 43–63. (doi:10.1089/cmb.2006.13.43)

70. Bujnicki JM. 2002 Sequence permutations in the molecular evolution of DNA methyltransferases. *BMC Evol. Biol.* **2**, 3. (doi:10.1186/1471-2148-2-3)

71. Jeltsch A. 1999 Circular permutations in the molecular evolution of DNA methyltransferases. *J. Mol. Evol.* **49**, 161–164. (doi:10.1007/PL00006529)

72. Kelil A, Wang S, Brzezinski R, Fleury A. 2007 CLUSS: clustering of protein sequences based on a new similarity measure. *BMC Bioinformatics* **8**, 286. (doi:10.1186/1471-2105-8-286)

73. Martin J, Anamika K, Srinivasan N. 2010 Classification of protein kinases on the basis of both kinase and non-kinase regions. *PLoS ONE* **5**, e12460. (doi:10.1371/journal.pone.0012460)

74. Lee B, Lee D. 2008 DAhunter: a web-based server that identifies homologous proteins by comparing domain architecture. *Nucleic Acids Res.* **36**, W60–W64. (doi:10.1093/nar/gkn172)

75. Lin K, Zhu L, Zhang DY. 2006 An initial strategy for comparing proteins at the domain architecture level. *Bioinformatics* **22**, 2081–2086. (doi:10.1093/bioinformatics/btl366)

76. Terrapon N, Weiner J, Grath S, Moore AD, Bornberg-Bauer E. 2013 Rapid similarity search of proteins using alignments of domain arrangements. *Bioinformatics* **30**, 274–281. (doi:10.1093/bioinformatics/btt379)

77. Weiner 3rd J, Thomas G, Bornberg-Bauer E. 2005 Rapid motif-based prediction of circular permutations in multi-domain proteins. *Bioinformatics* **21**, 932–937. (doi:10.1093/bioinformatics/bti085)

78. Edgar RC, Sjolander K. 2004 A comparison of scoring functions for protein sequence profile alignment. *Bioinformatics* **20**, 1301–1308. (doi:10.1093/bioinformatics/bth090)

79. Hendlich M, Lackner P, Weitckus S, Floeckner H, Froschauer R, Gottsbacher K, Casari G, Sippl MJ. 1990 Identification of native protein folds amongst a large number of incorrect models. The calculation of low energy conformations from potentials of mean force. *J. Mol. Biol.* **216**, 167–180. (doi:10.1016/S0022-2836(05)80068-3)

80. Sippl MJ. 1990 Calculation of conformational ensembles from potentials of mean force. An approach to the knowledge-based prediction of local structures in globular proteins. *J. Mol. Biol.* **213**, 859–883. (doi:10.1016/S0022-2836(05) 80269-4)

81. Godzik A, Kolinski A, Skolnick J. 1992 Topology fingerprint approach to the inverse protein folding problem. *J. Mol. Biol.* **227**, 227–238. (doi:10.1016/0022-2836(92)90693-E)

82. Godzik A, Skolnick J. 1992 Sequence-structure matching in globular proteins: application to supersecondary and tertiary structure determination. *Proc. Natl Acad. Sci. USA* **89**, 12 098–12 102. (doi:10.1073/pnas.89.24.12098)

83. Jones DT, Taylor WR, Thornton JM. 1992 A new approach to protein fold recognition. *Nature* **358**, 86–89. (doi:10.1038/358086a0)

84. Sippl MJ, Weitckus S. 1992 Detection of native-like models for amino acid sequences of unknown three-dimensional structure in a data base of known protein conformations. *Proteins* **13**, 258–271. (doi:10.1002/prot.340130308)

85. Bryant SH. 1996 Evaluation of threading specificity and accuracy. *Proteins* **26**, 172–185. (doi:10.1002/(SICI)1097-0134(199610)26:2<172::AID-PROT7>3.0.CO;2-I)

86. Xu Y, Xu D, Uberbacher EC. 1998 An efficient computational method for globally optimal threading. *J. Comput. Biol.* **5**, 597–614. (doi:10.1089/cmb.1998.5.597)

87. Xu J, Li M, Kim D, Xu Y. 2003 RAPTOR: optimal protein threading by linear programming. *J. Bioinform. Comput. Biol.* **1**, 95–117. (doi:10.1142/S0219720003000186)

88. Ma J, Wang S, Zhao F, Xu J. 2013 Protein threading using context-specific alignment potential. *Bioinformatics* **29**, i257–i265. (doi:10.1093/bioinformatics/btt210)

89. Fischer D. 2000 Hybrid fold recognition: combining sequence derived properties with evolutionary information. *Pac. Symp. Biocomput.* **2000**, 119–130.

90. Rice DW, Eisenberg D. 1997 A 3D-1D substitution matrix for protein fold recognition that includes predicted secondary structure of the sequence. *J. Mol. Biol.* **267**, 1026–1038. (doi:10.1006/jmbi.1997.0924)

91. Shi J, Blundell TL, Mizuguchi K. 2001 FUGUE: sequence-structure homology recognition using environment-specific substitution tables and structure-dependent gap penalties. *J. Mol. Biol.* **310**, 243–257. (doi:10.1006/jmbi.2001.4762)

92. Torda AE, Procter JB, Huber T. 2004 Wurst: a protein threading server with a structural scoring function, sequence profiles and optimized substitution matrices. *Nucleic Acids Res.* **32**, W532–W535. (doi:10.1093/nar/gkh357)

93. Mooney C, Pollastri G. 2009 Beyond the Twilight Zone: automated prediction of structural properties of proteins by recursive neural networks and remote homology information. *Proteins* **77**, 181–190. (doi:10.1002/prot.22429)

94. Rost B, Schneider R, Sander C. 1997 Protein fold recognition by prediction-based threading. *J. Mol. Biol.* **270**, 471–480. (doi:10.1006/jmbi.1997.1101)

95. Kelley LA, Sternberg MJ. 2009 Protein structure prediction on the Web: a case study using the Phyre server. *Nat. Protoc.* **4**, 363–371. (doi:10.1038/nprot.2009.2)

96. Przybylski D, Rost B. 2004 Improving fold recognition without folds. *J. Mol. Biol.* **341**, 255–269. (doi:10.1016/j.jmb.2004.05.041)

97. Rost B, Sander C. 1995 Progress of 1D protein structure prediction at last. *Proteins* **23**, 295–300. (doi:10.1002/prot.340230304)

98. Wu S, Zhang Y. 2007 LOMETS: a local meta-threading-server for protein structure prediction. *Nucleic Acids Res.* **35**, 3375–3382. (doi:10.1093/nar/gkm251)

99. Shah PK, Aloy P, Bork P, Russell RB. 2005 Structural similarity to bridge sequence space: finding new families on the bridges. *Protein Sci.* **14**, 1305–1314. (doi:10.1110/ps.041187405)

100. Sillitoe I, Dibley M, Bray J, Addou S, Orengo C. 2005 Assessing strategies for improved superfamily recognition. *Protein Sci.* **14**, 1800–1810. (doi:10.1110/ps.041056105)

101. Skolnick J, Kihara D. 2001 Defrosting the frozen approximation: PROSPECTOR—a new approach to threading. *Proteins* **42**, 319–331. (doi:10.1002/1097-0134(20010215)42:3<319::AID-PROT30>3.0.CO;2-A)

102. Skolnick J, Kihara D, Zhang Y. 2004 Development and large scale benchmark testing of the PROSPECTOR_3 threading algorithm. *Proteins* **56**, 502–518. (doi:10.1002/prot.20106)

103. Teichert F, Minning J, Bastolla U, Porto M. 2010 High quality protein sequence alignment by combining structural profile prediction and profile alignment using SABER-TOOTH. *BMC Bioinformatics* **11**, 251. (doi:10.1186/1471-2105-11-251)

104. Wang Y, Sadreyev RI, Grishin NV. 2009 PROCAIN: protein profile comparison with assisting information. *Nucleic Acids Res.* **37**, 3522–3530. (doi:10.1093/nar/gkp212)

105. Zhang W, Liu S, Zhou Y. 2008 SP5: improving protein fold recognition by using torsion angle profiles and profile-based gap penalty model. *PLoS ONE* **3**, e2325. (doi:10.1371/journal.pone.0002325)

106. Zhou H, Zhou Y. 2005 Fold recognition by combining sequence profiles derived from evolution and from depth-dependent structural alignment of fragments. *Proteins* **58**, 321–328. (doi:10.1002/prot.20308)

107. Jones DT. 1999 GenTHREADER: an efficient and reliable protein fold recognition method for genomic sequences. *J. Mol. Biol.* **287**, 797–815. (doi:10.1006/jmbi.1999.2583)

108. Zhang Y. 2008 I-TASSER server for protein 3D structure prediction. *BMC Bioinformatics* **9**, 40. (doi:10.1186/1471-2105-9-40)

109. Brylinski M. 2013 The utility of artificially evolved sequences in protein threading and fold recognition. *J. Theor. Biol.* **328**, 77–88. (doi:10.1016/j.jtbi.2013.03.018)

110. Pauling L, Corey RB, Branson HR. 1951 The structure of proteins; two hydrogen-bonded helical configurations of the polypeptide chain. *Proc. Natl Acad. Sci. USA* **37**, 205–211. (doi:10.1073/pnas.37.4.205)

111. Geourjon C, Combet C, Blanchet C, Deleage G. 2001 Identification of related proteins with weak sequence identity using secondary structure information. *Protein Sci.* **10**, 788–797. (doi:10.1110/ps.30001)

112. Westhead DR, Thornton JM. 1998 Protein structure prediction. *Curr. Opin. Biotechnol.* **9**, 383–389. (doi:10.1016/S0958-1669(98)80012-8)

113. Wu S, Zhang Y. 2008 MUSTER: improving protein sequence profile–profile alignments by using multiple sources of structure information. *Proteins* **72**, 547–556. (doi:10.1002/prot.21945)

114. Yang Y, Faraggi E, Zhao H, Zhou Y. 2011 Improving protein fold recognition and template-based modeling by employing probabilistic-based matching between predicted one-dimensional structural properties of query and corresponding native properties of templates. *Bioinformatics* **27**, 2076–2082. (doi:10.1093/bioinformatics/btr350)

115. Jones DT. 1999 Protein secondary structure prediction based on position-specific scoring matrices. *J. Mol. Biol.* **292**, 195–202. (doi:10.1006/jmbi.1999.3091)

116. Guo J, Chen H, Sun Z, Lin Y. 2004 A novel method for protein secondary structure prediction using dual-layer SVM and profiles. *Proteins* **54**, 738–743. (doi:10.1002/prot.10634)

117. Bondugula R, Xu D. 2007 MUPRED: a tool for bridging the gap between template based methods and sequence profile based methods for protein secondary structure prediction. *Proteins* **66**, 664–670. (doi:10.1002/prot.21177)

118. Cheng H, Sen TZ, Jernigan RL, Kloczkowski A. 2007 Consensus Data Mining (CDM) Protein Secondary Structure Prediction Server: combining GOR V and fragment database mining (FDM). *Bioinformatics* **23**, 2628–2630. (doi:10.1093/bioinformatics/btm379)

119. Karypis G. 2006 YASSPP: better kernels and coding schemes lead to improvements in protein secondary structure prediction. *Proteins* **64**, 575–586. (doi:10.1002/prot.21036)

120. Nguyen MN, Rajapakse JC. 2007 Prediction of protein secondary structure with two-stage multi-class SVMs. *Int. J. Data Min. Bioinform.* **1**, 248–269. (doi:10.1504/IJDMB.2007.011612)

121. Pollastri G, McLysaght A. 2005 Porter: a new, accurate server for protein secondary structure prediction. *Bioinformatics* **21**, 1719–1720. (doi:10.1093/bioinformatics/bti203)

122. Rost B, Eyrich VA. 2001 EVA: large-scale analysis of secondary structure prediction. *Proteins* **45**, 192–199. (doi:10.1002/prot.10051)

123. Rost B. 2001 Review: protein secondary structure prediction continues to rise. *J. Struct. Biol.* **134**, 204–218. (doi:10.1006/jsbi.2001.4336)

124. de Brevern AG, Benros C, Hazout S. 2005 Structural alphabet: from a local point of view to a global description of protein 3D structures. In *Bioinformatics: new research* (ed. PV Yan), pp. 128–187. New York, NY: Nova Publishers.

125. Martin J, Letellier G, Marin A, Taly J-F, de Brevern AG, Gibrat J-F. 2005 Protein secondary structure assignment revisited: a detailed analysis of different assignment methods. *BMC Struct. Biol.* **5**, 17. (doi:10.1186/1472-6807-5-17)

126. Tyagi M, Bornot A, Offmann B, de Brevern AG. 2009 Analysis of loop boundaries using different local structure assignment methods. *Protein Sci.* **18**, 1869–1881. (doi:10.1002/pro.198)

127. Shahsavar A, Erfani Moghaddam M, Antonyuk SV, Khajeh K, Naderi-Manesh H. In preparation. Crystal structures of methylglyoxal synthase from Thermus sp. GH5 in the open and closed conformational states provide insight into the mechanism of allosteric regulation.

128. Kabsch W, Sander C. 1983 Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers* **22**, 2577–2637. (doi:10.1002/bip.360221211)

129. Rost B, Sander C. 1993 Improved prediction of protein secondary structure by use of sequence profiles and neural networks. *Proc. Natl Acad. Sci. USA* **90**, 7558–7562. (doi:10.1073/pnas.90.16.7558)

130. Pollastri G, Przybylski D, Rost B, Baldi P. 2002 Improving the prediction of protein secondary structure in three and eight classes using recurrent neural networks and profiles. *Proteins* **47**, 228–235. (doi:10.1002/prot.10082)

131. Lin K, Simossis VA, Taylor WR, Heringa J. 2005 A simple and fast secondary structure prediction method using hidden neural networks. *Bioinformatics* **21**, 152–159. (doi:10.1093/bioinformatics/bth487)

132. Kruus E, Thumfort P, Tang C, Wingreen NS. 2005 Gibbs sampling and helix-cap motifs. *Nucleic Acids Res.* **33**, 5343–5353. (doi:10.1093/nar/gki842)

133. Cole C, Barber JD, Barton GJ. 2008 The Jpred 3 secondary structure prediction server. *Nucleic Acids Res.* **36**, W197–W201. (doi:10.1093/nar/gkn238)

134. Duan M, Huang M, Ma C, Li L, Zhou Y. 2008 Position-specific residue preference features around the ends of helices and strands and a novel strategy for the prediction of secondary structures. *Protein Sci.* **17**, 1505–1512. (doi:10.1110/ps.035691.108)

135. Kountouris P, Hirst JD. 2009 Prediction of backbone dihedral angles and protein secondary structure using support vector machines. *BMC Bioinformatics* **10**, 437. (doi:10.1186/1471-2105-10-437)

136. Montgomerie S, Sundararaj S, Gallin WJ, Wishart DS. 2006 Improving the accuracy of protein secondary structure prediction using structural alignment. *BMC Bioinformatics* **7**, 301. (doi:10.1186/1471-2105-7-301)

137. Lin HN, Sung TY, Ho SY, Hsu WL. 2010 Improving protein secondary structure prediction based on short subsequences with local structure similarity. *BMC Genomics* **11**(Suppl. 4), S4. (doi:10.1186/1471-2164-11-S4-S4)

138. Zhang H, Zhang T, Chen K, Kedarisetti KD, Mizianty MJ, Bao Q, Stach W, Kurgan L. 2011 Critical assessment of high-throughput standalone methods for secondary structure prediction. *Brief. Bioinform.* **12**, 672–688. (doi:10.1093/bib/bbq088)

139. Ginalski K, Grishin NV, Godzik A, Rychlewski L. 2005 Practical lessons from protein structure prediction. *Nucleic Acids Res.* **33**, 1874–1891. (doi:10.1093/nar/gki327)

140. Soding J, Biegert A, Lupas AN. 2005 The HHpred interactive server for protein homology detection and structure prediction. *Nucleic Acids Res.* **33**, W244–W248. (doi:10.1093/nar/gki408)

141. Shi Y, Zhou J, Arndt D, Wishart DS, Lin G. 2008 Protein contact order prediction from primary sequences. *BMC Bioinformatics* **9**, 255. (doi:10.1186/1471-2105-9-255)

142. Fontana P, Bindewald E, Toppo S, Velasco R, Valle G, Tosatto SCE. 2005 The SSEA server for protein secondary structure alignment. *Bioinformatics* **21**, 393–395. (doi:10.1093/bioinformatics/bti013)

143. Guillet V, Ohta N, Cabantous S, Newton A, Samama J-P. 2002 Crystallographic and biochemical studies of DivK reveal novel features of an essential response regulator in *Caulobacter crescentus*. *J. Biol. Chem.* **277**, 42 003–42 010. (doi:10.1074/jbc.M204789200)

144. De La Cruz X, Thornton JM. 1999 Factors limiting the performance of prediction-based fold recognition methods. *Protein Sci.* **8**, 750–759. (doi:10.1110/ps.8.4.750)

145. Venkatachalam CM. 1968 Stereochemical criteria for polypeptides and proteins. V. Conformation of a system of three linked peptide units. *Biopolymers* **6**, 1425–1436. (doi:10.1002/bip.1968.360061006)

146. Hutchinson EG, Thornton JM. 1994 A revised set of potentials for beta-turn formation in proteins. *Protein Sci.* **3**, 2207–2216. (doi:10.1002/pro.5560031206)

147. Mansiaux Y, Joseph AP, Gelly JC, de Brevern AG. 2011 Assignment of PolyProline II conformation and analysis of sequence–structure relationship. *PLoS ONE* **6**, e18401. (doi:10.1371/journal.pone.0018401)

148. Offmann B, Tyagi M, de Brevern AG. 2007 Local protein structures. *Curr. Bioinform.* **3**, 165–202. (doi:10.2174/157489307781662105)

149. Joseph AP, Bornot A, de Brevern AG. 2010 Local structural alphabets. In *Introduction to protein structure prediction: methods and algorithms* (eds H Rangwala, G Karypis), pp. 75–106. Hoboken, NJ: John Wiley & Sons, Inc.

150. Bystroff C, Baker D. 1998 Prediction of local structure in proteins using a library of sequence–structure motifs. *J. Mol. Biol.* **281**, 565–577. (doi:10.1006/jmbi.1998.1943)

151. Camproux AC, de Brevern AG, Hazout S, Tufféry P. 2001 Exploring the use of a structural alphabet for structural prediction of protein loops. *Theor. Chem. Acc.* **106**, 28–35. (doi:10.1007/s002140100261)

152. Chen B, Johnson M. 2009 Protein local 3D structure prediction by Super Granule Support Vector Machines (Super GSVM). *BMC Bioinformatics* **10**(Suppl. 11), S15. (doi:10.1186/1471-2105-10-S11-S15)

153. de Brevern AG, Etchebest C, Hazout S. 2000 Bayesian probabilistic approach for predicting backbone structures in terms of protein blocks. *Proteins* **41**, 271–287. (doi:10.1002/1097-0134(20001115)41:3<271::AID-PROT10>3.0.CO;2-Z)

154. Fetrow JS, Palumbo MJ, Berg G. 1997 Patterns, structures, and amino acid frequencies in structural building blocks, a protein secondary structure classification scheme. *Proteins* **27**, 249–271. (doi:10.1002/(SICI)1097-0134(199702)27:2<249::AID-PROT11>3.0.CO;2-M)

155. Hunter CG, Subramaniam S. 2003 Protein fragment clustering and canonical local shapes. *Proteins* **50**, 580–588. (doi:10.1002/prot.10309)

156. Rooman MJ, Rodriguez J, Wodak SJ. 1990 Relations between protein sequence and structure and their significance. *J. Mol. Biol.* **213**, 337–350. (doi:10.1016/S0022-2836(05)80195-0)

157. Schuchhardt J, Schneider G, Reichelt J, Schomburg D, Wrede P. 1996 Local structural motifs of protein backbones are classified by self-organizing neural networks. *Protein Eng.* **9**, 833–842. (doi:10.1093/protein/9.10.833)

158. Hunter CG, Subramaniam S. 2003 Protein local structure prediction from sequence. *Proteins* **50**, 572–579. (doi:10.1002/prot.10310)

159. Dong Q, Wang X, Lin L, Wang Y. 2008 Analysis and prediction of protein local structure based on structure alphabets. *Proteins* **72**, 163–172. (doi:10.1002/prot.21904)

160. Rangwala H, Kauffman C, Karypis G. 2009 svmPRAT: SVM-based protein residue annotation toolkit. *BMC Bioinformatics* **10**, 439. (doi:10.1186/1471-2105-10-439)

161. Sander O, Sommer I, Lengauer T. 2006 Local protein structure prediction using discriminative models. *BMC Bioinformatics* **7**, 14. (doi:10.1186/1471-2105-7-14)

162. Yang AS, Wang LY. 2003 Local structure prediction with local structure-based sequence profiles. *Bioinformatics* **19**, 1267–1274. (doi:10.1093/bioinformatics/btg151)

163. Bystroff C, Thorsson V, Baker D. 2000 HMMSTR: a hidden Markov model for local sequence-structure correlations in proteins. *J. Mol. Biol.* **301**, 173–190. (doi:10.1006/jmbi.2000.3837)

164. Etchebest C, Benros C, Hazout S, de Brevern AG. 2005 A structural alphabet for local protein structures: improved prediction methods. *Proteins* **59**, 810–827. (doi:10.1002/prot.20458)

165. de Brevern AG, Benros C, Gautier R, Valadie H, Hazout S, Etchebest C. 2004 Local backbone structure prediction of proteins. *In Silico Biol.* **4**, 381–386.

166. de Brevern AG, Etchebest C, Benros C, Hazout S. 2007 ‘Pinning strategy’: a novel approach for predicting the backbone structure in terms of protein blocks from sequence. *J. Biosci.* **32**, 51–72. (doi:10.1007/s12038-007-0006-3)

167. Zimmermann O, Hansmann UH. 2008 LOCUSTRA: accurate prediction of local protein structure using a two-layer support vector machine approach. *J. Chem. Inf. Model.* **48**, 1903–1908. (doi:10.1021/ci800178a)

168. Li Q, Zhou C, Liu H. 2009 Fragment-based local statistical potentials derived by combining an alphabet of protein local structures with secondary structures and solvent accessibilities. *Proteins* **74**, 820–836. (doi:10.1002/prot.22191)

169. de Brevern AG, Hazout S. 2003 ‘Hybrid protein model’ for optimally defining 3D protein structure fragments. *Bioinformatics* **19**, 345–353. (doi:10.1093/bioinformatics/btf859)

170. Bornot A, Etchebest C, de Brevern AG. 2009 A new prediction strategy for long local protein structures using an original description. *Proteins* **76**, 570–587. (doi:10.1002/prot.22370)

171. Bornot A, Etchebest C, de Brevern AG. 2011 Predicting protein flexibility through the prediction of local structures. *Proteins* **79**, 839–852. (doi:10.1002/prot.22922)

172. Ramachandran GN, Ramakrishnan C, Sasisekharan V. 1963 Stereochemistry of polypeptide chain configurations. *J. Mol. Biol.* **7**, 95–99. (doi:10.1016/S0022-2836(63)80023-6)

173. Kuang R, Leslie CS, Yang AS. 2004 Protein backbone angle prediction with machine learning approaches. *Bioinformatics* **20**, 1612–1621. (doi:10.1093/bioinformatics/bth136)

174. Zimmermann O, Hansmann UH. 2006 Support vector machines for prediction of dihedral angle regions. *Bioinformatics* **22**, 3009–3015. (doi:10.1093/bioinformatics/btl489)

175. Helles G, Fonseca R. 2009 Predicting dihedral angle probability distributions for protein coil residues from primary sequence using neural networks. *BMC Bioinformatics* **10**, 338. (doi:10.1186/1471-2105-10-338)

176. Wood MJ, Hirst JD. 2005 Protein secondary structure prediction with dihedral angles. *Proteins* **59**, 476–481. (doi:10.1002/prot.20435)

177. Wu S, Zhang Y. 2008 ANGLOR: a composite machine-learning algorithm for protein backbone torsion angle prediction. *PLoS ONE* **3**, e3400. (doi:10.1371/journal.pone.0003400)

178. Xue B, Dor O, Faraggi E, Zhou Y. 2008 Real-value prediction of backbone torsion angles. *Proteins* **72**, 427–433. (doi:10.1002/prot.21940)

179. Faraggi E, Xue B, Zhou Y. 2009 Improving the prediction accuracy of residue solvent accessibility and real-value backbone torsion angles of proteins by guided-learning through a two-layer neural network. *Proteins* **74**, 847–856. (doi:10.1002/prot.22193)

180. Karchin R, Cline M, Mandel-Gutfreund Y, Karplus K. 2003 Hidden Markov models that use predicted local structure for fold recognition: alphabets of backbone geometry. *Proteins* **51**, 504–514. (doi:10.1002/prot.10369)

181. Hu Y, Dong X, Wu A, Cao Y, Tian L, Jiang T. 2011 Incorporation of local structural preference potential improves fold recognition. *PLoS ONE* **6**, e17215. (doi:10.1371/journal.pone.0017215)

182. Karplus K, Barrett C, Hughey R. 1998 Hidden Markov models for detecting remote protein homologies. *Bioinformatics* **14**, 846–856. (doi:10.1093/bioinformatics/14.10.846)

183. Zhang Y, Arakaki AK, Skolnick J. 2005 TASSER: an automated method for the prediction of protein tertiary structures in CASP6. *Proteins* **61**(Suppl. 7), 91–98. (doi:10.1002/prot.20724)

184. Zhang Y, Kolinski A, Skolnick J. 2003 TOUCHSTONE II: a new approach to ab initio protein structure prediction. *Biophys. J.* **85**, 1145–1164. (doi:10.1016/S0006-3495(03)74551-2)

185. Grishin NV. 2001 Fold change in evolution of protein structures. *J. Struct. Biol.* **134**, 167–185. (doi:10.1006/jsbi.2001.4335)

186. Chivian D *et al*. 2003 Automated prediction of CASP-5 structures using the Robetta server. *Proteins* **53**(Suppl. 6), 524–533. (doi:10.1002/prot.10529)

187. Rohl CA, Strauss CE, Chivian D, Baker D. 2004 Modeling structurally variable regions in homologous proteins with rosetta. *Proteins* **55**, 656–677. (doi:10.1002/prot.10629)

188. Simons KT, Kooperberg C, Huang E, Baker D. 1997 Assembly of protein tertiary structures from fragments with similar local sequences using simulated annealing and Bayesian scoring functions. *J. Mol. Biol.* **268**, 209–225. (doi:10.1006/jmbi.1997.0959)

189. Gullotto D, Nolassi MS, Bernini A, Spiga O, Niccolai N. 2012 Probing the protein space for extending the detection of weak homology folds. *J. Theor. Biol.* **320**, 152–158. (doi:10.1016/j.jtbi.2012.12.005)

190. Sadreyev RI, Tang M, Kim BH, Grishin NV. 2009 COMPASS server for homology detection: improved statistical accuracy, speed and functionality. *Nucleic Acids Res.* **37**, W90–W94. (doi:10.1093/nar/gkp360)

191. Davtyan A, Schafer NP, Zheng W, Clementi C, Wolynes PG, Papoian GA. 2012 AWSEM-MD: protein structure prediction using coarse-grained physical potentials and bioinformatically based local structure biasing. *J. Phys. Chem. B* **116**, 8494–8503. (doi:10.1021/jp212541y)

192. Hvidsten TR, Kryshtafovych A, Komorowski J, Fidelis K. 2003 A novel approach to fold recognition using sequence-derived properties from sets of structurally similar local fragments of proteins. *Bioinformatics* **19**(Suppl. 2), ii81–ii91. (doi:10.1093/bioinformatics/btg1064)

193. Jones DT, McGuffin LJ. 2003 Assembling novel protein folds from super-secondary structural fragments. *Proteins* **53**(Suppl. 6), 480–485. (doi:10.1002/prot.10542)

194. Karplus K, Karchin R, Draper J, Casper J, Mandel-Gutfreund Y, Diekhans M, Hughey R. 2003 Combining local-structure, fold-recognition, and new fold methods for protein structure prediction. *Proteins* **53**(Suppl. 6), 491–496. (doi:10.1002/prot.10540)

195. Kim DE, Chivian D, Baker D. 2004 Protein structure prediction and analysis using the Robetta server. *Nucleic Acids Res.* **32**, W526–W531. (doi:10.1093/nar/gkh468)

196. Lee J, Kim SY. 2005 Protein structure prediction based on fragment assembly and parameter optimization. *Biophys. Chem.* **115**, 209–214. (doi:10.1016/j.bpc.2004.12.046)

197. Kosinski J *et al*. 2005 FRankenstein becomes a cyborg: the automatic recombination and realignment of fold recognition models in CASP6. *Proteins* **61**(Suppl. 7), 106–113. (doi:10.1002/prot.20726)

198. Zhang Y, Skolnick J. 2004 Automated structure prediction of weakly homologous proteins on a genomic scale. *Proc. Natl Acad. Sci. USA* **101**, 7594–7599. (doi:10.1073/pnas.0305695101)

199. Handl J, Knowles J, Vernon R, Baker D, Lovell SC. 2011 The dual role of fragments in fragment-assembly methods for de novo protein structure prediction. *Proteins* **80**, 490–504. (doi:10.1002/prot.23215)

200. Ghozlane A, Joseph AP, Bornot A, de Brevern AG. 2009 Analysis of protein chameleon sequence characteristics. *Bioinformation* **3**, 367–369. (doi:10.6026/97320630003367)

201. Han KF, Baker D. 1996 Global properties of the mapping between local amino acid sequence and local structure in proteins. *Proc. Natl Acad. Sci. USA* **93**, 5814–5818. (doi:10.1073/pnas.93.12.5814)

202. Kabsch W, Sander C. 1984 On the use of sequence homologies to predict protein structure: identical pentapeptides can have completely different conformations. *Proc. Natl Acad. Sci. USA* **81**, 1075–1078. (doi:10.1073/pnas.81.4.1075)

203. Kister AE, Potapov V. 2011 Amino acid distribution rules predict protein fold. *Biochem. Soc. Trans.* **41**, 616–619. (doi:10.1042/BST20120308)

204. Minor Jr DL, Kim PS. 1996 Context-dependent secondary structure formation of a designed protein sequence. *Nature* **380**, 730–734. (doi:10.1038/380730a0)

205. Adamczak R, Porollo A, Meller J. 2005 Combining prediction of secondary structure and solvent accessibility in proteins. *Proteins* **59**, 467–475. (doi:10.1002/prot.20441)

206. Macdonald JR, Johnson Jr WC. 2001 Environmental features are important in determining protein secondary structure. *Protein Sci.* **10**, 1172–1177. (doi:10.1110/ps.420101)

207. Zhu ZY, Blundell TL. 1996 The use of amino acid patterns of classified helices and strands in secondary structure prediction. *J. Mol. Biol.* **260**, 261–276. (doi:10.1006/jmbi.1996.0397)

208. Momen-Roknabadi A, Sadeghi M, Pezeshk H, Marashi SA. 2008 Impact of residue accessible surface area on the prediction of protein secondary structures. *BMC Bioinformatics* **9**, 357. (doi:10.1186/1471-2105-9-357)

209. Gong S, Blundell TL. 2008 Discarding functional residues from the substitution table improves predictions of active sites within three-dimensional structures. *PLoS Comput. Biol.* **4**, e1000179. (doi:10.1371/journal.pcbi.1000179)

210. Bertoncini CW, Jung YS, Fernandez CO, Hoyer W, Griesinger C, Jovin TM, Zweckstetter M. 2005 Release of long-range tertiary interactions potentiates aggregation of natively unstructured alpha-synuclein. *Proc. Natl Acad. Sci. USA* **102**, 1430–1435. (doi:10.1073/pnas.0407146102)

211. Fedyukina DV, Rajagopalan S, Sekhar A, Fulmer EC, Eun YJ, Cavagnero S. 2010 Contribution of long-range interactions to the secondary structure of an unfolded globin. *Biophys. J.* **99**, L37–L39. (doi:10.1016/j.bpj.2010.06.038)

212. Ruvinsky AM, Vakser IA. 2010 Sequence composition and environment effects on residue fluctuations in protein structures. *J. Chem. Phys.* **133**, 155101. (doi:10.1063/1.3498743)

213. Crooks GE, Brenner SE. 2004 Protein secondary structure: entropy, correlations and prediction. *Bioinformatics* **20**, 1603–1611. (doi:10.1093/bioinformatics/bth132)

214. Kihara D. 2005 The effect of long-range interactions on the secondary structure formation of proteins. *Protein Sci.* **14**, 1955–1963. (doi:10.1110/ps.051479505)

215. Gendoo DM, Harrison PM. 2011 Discordant and chameleon sequences: their distribution and implications for amyloidogenicity. *Protein Sci.* **20**, 567–579. (doi:10.1002/pro.590)

216. de Brevern AG, Bornot A, Craveur P, Etchebest C, Gelly JC. 2012 PredyFlexy: flexibility and local structure prediction from sequence. *Nucleic Acids Res.* **40**, W317–W322. (doi:10.1093/nar/gks482)

217. Schlessinger A, Yachdav G, Rost B. 2006 PROFbval: predict flexible and rigid residues in proteins. *Bioinformatics* **22**, 891–893. (doi:10.1093/bioinformatics/btl032)

218. Reitzer R, Gruber K, Jogl G, Wagner UG, Bothe H, Buckel W, Kratky C. 1999 Glutamate mutase from *Clostridium cochlearium*: the structure of a coenzyme B_{12}-dependent enzyme provides new mechanistic insights. *Structure* **7**, 891–902. (doi:10.1016/S0969-2126(99)80116-6)

219. Rost B, Yachdav G, Liu J. 2004 The PredictProtein server. *Nucleic Acids Res.* **32**(Suppl. 2), W321–W326. (doi:10.1093/nar/gkh377)

220. Ishida T, Kinoshita K. 2007 PrDOS: prediction of disordered protein regions from amino acid sequence. *Nucleic Acids Res.* **35**(Suppl. 2), W460–W464. (doi:10.1093/nar/gkm363)

221. Paila U, Kondam R, Ranjan A. 2008 Genome bias influences amino acid choices: analysis of

amino acid substitution and re-compilation of substitution matrices exclusive to an AT-biased genome. *Nucleic Acids Res.* **36**, 6664–6675. (doi:10.1093/nar/gkn635)

222. Marashi SA, Behrouzi R, Pezeshk H. 2007 Adaptation of proteins to different environments: a comparison of proteome structural properties in *Bacillus subtilis* and *Escherichia coli*. *J. Theor. Biol.* **244**, 127–132. (doi:10.1016/j.jtbi.2006.07.021)

223. de Brevern AG, Joseph AP. 2011 Species specific amino acid sequence-protein local structure relationships: an analysis in the light of a structural alphabet. *J. Theor. Biol.* **276**, 209–217. (doi:10.1016/j.jtbi.2011.01.047)

224. Sadreyev RI, Wang Y, Grishin NV. 2009 Considering scores between unrelated proteins in the search database improves profile comparison. *BMC Bioinformatics* **10**, 399. (doi:10.1186/1471-2105-10-399)

225. Costantini S, Colonna G, Facchiano AM. 2006 Amino acid propensities for secondary structures are influenced by the protein structural class. *Biochem. Biophys. Res. Commun.* **342**, 441–451. (doi:10.1016/j.bbrc.2006.01.159)

226. Bhardwaj N, Langlois RE, Zhao G, Lu H. 2005 Kernel-based machine learning protocol for predicting DNA-binding proteins. *Nucleic Acids Res.* **33**, 6486–6493. (doi:10.1093/nar/gki949)

227. Fariselli P, Finelli M, Rossi I, Amico M, Zauli A, Martelli PL, Casadio R. 2005 TRAMPLE: the transmembrane protein labelling environment. *Nucleic Acids Res.* **33**, W198–W201. (doi:10.1093/nar/gki440)

228. Battey JN, Kopp J, Bordoli L, Read RJ, Clarke ND, Schwede T. 2007 Automated server predictions in CASP7. *Proteins* **69**(Suppl. 8), 68–82. (doi:10.1002/prot.21761)

229. Xu D, Zhang Y. 2012 *Ab initio* protein structure assembly using continuous structure fragments and optimized knowledge-based force field. *Proteins* **80**, 1715–1735. (doi:10.1002/prot.24065)

230. Wallner B, Larsson P, Elofsson A. 2007 Pcons.net: protein structure prediction meta server. *Nucleic Acids Res.* **35**, W369–W374. (doi:10.1093/nar/gkm319)

231. Zhou H, Skolnick J. 2009 Protein structure prediction by pro-Sp3-TASSER. *Biophys. J.* **96**, 2119–2127. (doi:10.1016/j.bpj.2008.12.3898)

232. Li SC, Bu D, Xu J, Li M. 2008 Fragment-HMM: a new approach to protein structure prediction. *Protein Sci.* **17**, 1925–1934. (doi:10.1110/ps.036442.108)

233. Pei J, Grishin NV. 2004 Combining evolutionary and structural information for local protein structure prediction. *Proteins* **56**, 782–794. (doi:10.1002/prot.20158)

234. Peng J, Xu J. 2009 Boosting protein threading accuracy. In *Research in computational molecular biology* (ed. S Batzoglou). Lecture Notes in Computer Science, vol. 5541, pp. 31–45. Berlin, Germany: Springer. (doi:10.1007/978-3-642-02008-7_3)

235. Mirny LA, Shakhnovich EI. 1998 Protein structure prediction by threading. Why it works and why it does not. *J. Mol. Biol.* **283**, 507–526. (doi:10.1006/jmbi.1998.2092)

236. Bennett-Lovsey RM, Herbert AD, Sternberg MJ, Kelley LA. 2008 Exploring the extremes of sequence/structure space with ensemble fold recognition in the program Phyre. *Proteins* **70**, 611–625. (doi:10.1002/prot.21688)

237. Douguet D, Labesse G. 2001 Easier threading through web-based comparisons and cross-validations. *Bioinformatics* **17**, 752–753. (doi:10.1093/bioinformatics/17.8.752)

238. Ginalski K, Elofsson A, Fischer D, Rychlewski L. 2003 3D-Jury: a simple approach to improve protein structure predictions. *Bioinformatics* **19**, 1015–1018. (doi:10.1093/bioinformatics/btg124)

239. Kurowski MA, Bujnicki JM. 2003 GeneSilico protein structure prediction meta-server. *Nucleic Acids Res.* **31**, 3305–3307. (doi:10.1093/nar/gkg557)

240. Wallner B, Elofsson A. 2003 Can correct protein models be identified? *Protein Sci.* **12**, 1073–1086. (doi:10.1110/ps.0236803)

241. Wang Z, Eickholt J, Cheng J. 2010 MULTICOM: a multi-level combination approach to protein structure prediction and its assessments in CASP8. *Bioinformatics* **26**, 882–888. (doi:10.1093/bioinformatics/btq058)

242. Bujnicki JM. 2006 Protein-structure prediction by recombination of fragments. *Chembiochem* **7**, 19–27. (doi:10.1002/cbic.200500235)

243. Contreras-Moreira B, Fitzjohn PW, Bates PA. 2003 In silico protein recombination: enhancing template and sequence alignment selection for comparative protein modelling. *J. Mol. Biol.* **328**, 593–608. (doi:10.1016/S0022-2836(03)00309-7)

244. Siew N, Elofsson A, Rychlewski L, Fischer D. 2000 MaxSub: an automated measure for the assessment of protein structure prediction quality. *Bioinformatics* **16**, 776–785. (doi:10.1093/bioinformatics/16.9.776)

245. Zhang Y, Skolnick J. 2004 Tertiary structure predictions on a comprehensive benchmark of medium to large size proteins. *Biophys. J.* **87**, 2647–2655. (doi:10.1529/biophysj.104.045385)

246. John B, Sali A. 2003 Comparative protein structure modeling by iterative alignment, model building and model assessment. *Nucleic Acids Res.* **31**, 3982–3992. (doi:10.1093/nar/gkg460)

247. Liu T, Guerquin M, Samudrala R. 2008 Improving the accuracy of template-based predictions by mixing and matching between initial models. *BMC Struct. Biol.* **8**, 24. (doi:10.1186/1472-6807-8-24)

248. Wallner B, Fang H, Elofsson A. 2003 Automatic consensus-based fold recognition using Pcons, ProQ, and Pmodeller. *Proteins* **53**(Suppl. 6), 534–541. (doi:10.1002/prot.10536)

249. Wallner B, Elofsson A. 2005 Pcons5: combining consensus, structural evaluation and fold recognition scores. *Bioinformatics* **21**, 4248–4254. (doi:10.1093/bioinformatics/bti702)

250. Roche DB, Buenavista MT, Tetchner SJ, McGuffin LJ. 2011 The IntFOLD server: an integrated web resource for protein fold recognition, 3D model quality assessment, intrinsic disorder prediction, domain prediction and ligand binding site prediction. *Nucleic Acids Res.* **39**, W171–W176. (doi:10.1093/nar/gkr184)

251. Margelevicius M, Venclovas C. 2010 Detection of distant evolutionary relationships between protein families using theory of sequence profile–profile comparison. *BMC Bioinformatics* **11**, 89. (doi:10.1186/1471-2105-11-89)

252. Brylinski M, Lingam D. 2012 eThread: a highly optimized machine learning-based approach to meta-threading and the modeling of protein tertiary structures. *PLoS ONE* **7**, e50200. (doi:10.1371/journal.pone.0050200)

253. Zhang Y, Skolnick J. 2005 TM-align: a protein structure alignment algorithm based on the TM-score. *Nucleic Acids Res.* **33**, 2302–2309. (doi:10.1093/nar/gki524)