

PHD—an automatic mail server for protein secondary structure prediction

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Abstract

By the middle of 1993, > 30 000 protein sequences had been listed. For 1000 of these, the three-dimensional (tertiary) structure has been experimentally solved. Another 7000 can be modelled by homology. For the remaining 21 000 sequences, secondary structure prediction provides a rough estimate of structural features. Predictions in three states range between 35% (random) and 88% (homology modelling) overall accuracy. Using information about evolutionary conservation as contained in multiple sequence alignments, the secondary structure of 4700 protein sequences was predicted by the automatic e-mail server PHD. For proteins with at least one known homologue, the method has an expected overall three-state accuracy of 71.4% for proteins with at least one known homologue (evaluated on 126 unique protein chains).

Introduction

The number of known protein sequences (30 000 SWISSPROT release 25.0; Bairoch and Boeckmann, 1992) is growing much faster than that of known protein structures (1000 PDB; Bernstein *et al.*, 1977). About 300 of the known structures are unique in terms of detectable sequence homology (Hobohm *et al.*, 1992; U.Hobohm, personal communication). This situation makes theoretical predictions of structural features of proteins increasingly necessary.

Suppose one has a sequence of unknown structure (SOS) and wants to know as much as possible about the structure. How can theory help? If there is a protein with a similar sequence to SOS in the data bank of known structures, model building by homology allows the prediction of the structure of SOS with reasonable accuracy (Greer, 1980, 1981, 1990, 1991; Blundell *et al.*, 1987; Taylor and Orengo, 1989; Overington *et al.*, 1990; Summers and Karplus, 1990; Vriend and Sander, 1991; Holm and Sander, 1992b; Levitt, 1992; Taylor, 1992). If not, i.e. if the SOS belongs to the majority of the 75% of known sequences which do not have homologues among the known three-dimensional structures (Schneider and Sander, 1993), there is still a chance to model the fold. If the SOS is very short, molecular dynamics could perhaps help to fold it up (Karplus and Petsko, 1990; Jernigan, 1992; Abagyan and Totrov, 1993; Dill, 1993). If the SOS is too long, there still is a chance of finding the three-dimensional structure: one can try to thread

the SOS into a known structure, i.e. to find a protein of known structure which has no significant sequence similarity to SOS but is likely to have the same fold (Eisenberg and McLachlan, 1986; Baumann *et al.*, 1989; Overington *et al.*, 1990, 1992; Sippl, 1990; Crippen, 1991; Finkelstein and Reva, 1991; Lüthy *et al.*, 1991, 1992; Goldstein *et al.*, 1992; Holm and Sander, 1992a; Sippl and Weitckus, 1992; Ouzounis *et al.*, 1993; Stulz *et al.*, 1993). If this attempt also fails, prediction in three dimensions is no longer possible. One now has to revert to one dimension, i.e. to a prediction of one-dimensional strings of secondary structure assignment. More than 20 years of continuous effort to predict the secondary structure has led to the result that the performance is still far from 100% accurate; but 100% is not necessary. An important goal is that most secondary structure segments are predicted correctly. And for this there are promising methods.

How accurate is secondary structure prediction?

A random prediction of secondary structure in three states (helix, strand, rest—here termed loop) yields an overall per-residue accuracy of 35% (Rost *et al.*, 1993). [Note: for two state predictions such as helix/non-helix the random value is ~55% (Rost and Sander, 1993b)]. This value provides a lower limit for the evaluation of predictions. Early methods such as those of Chou and Fasman (1974), Robson *et al.* (Garnier *et al.*, 1978; Robson and Pain, 1971) and Lim (1974) scored 14–19% above the random level (Kabsch and Sander, 1983b). In the 1980s the accuracy increased to ~60–66% (Ptitsyn and Finkelstein, 1983; Levin *et al.*, 1986; Gibrat *et al.*, 1987; Biou *et al.*, 1988; Gascuel and Golmard, 1988; Levin and Garnier, 1988; Salzberg and Cost, 1992; Zhang *et al.*, 1992), i.e. 24–30% above the random level (Figure 1). Using profiles from multiple sequence alignments a system of neural networks (dubbed PHD) was the first method to achieve a performance accuracy >70% when cross-validated on >100 unique proteins (Rost and Sander, 1993a), i.e. >34% above the random level (Figure 1). What is a reasonable goal for accuracy in secondary structure prediction? An upper limit for a very accurate prediction is given by the accuracy to be expected if a three-dimensional homologue to the SOS were known, i.e. if its three-dimensional structure can be modelled by homology with reasonable accuracy. A comparison of 140 protein pairs of known structure shows that these have ~88% of their residues in identical secondary structure states: helix, strand or loop (Rost *et al.*, 1994).

But how good is the prediction for the test case SOS? Of course, the answer can only be estimated from the statistics over tests with proteins of known three-dimensional structure. For the 126 proteins used in the cross-validation test of PHD the standard deviation of the latest version was 9.5%, i.e. the prediction of SOS is likely to be $71.4 \pm 9.5\%$ accurate. Secondary structure predictions are successful in capturing the clichés contained in the data bank. So, the more unusual the SOS protein is compared to known structures, the less likely is a good prediction. Two recent examples of prediction failure are the phosphatidylinositol 3-OH kinase p58_{human} (Kohda *et al.*, 1993; Koyama *et al.*, 1993) and the anti-freeze protein type III anpc_{macam} (Sönnichsen *et al.*, 1993): both recently solved structures and both predicted at low accuracy of ~40%. Thus, there is a small but non-vanishing chance that the prediction for SOS is grossly wrong. A more encouraging

message is that the network prediction allows the identification of regions that are predicted with higher reliability. About 36% of all residues are predicted at an expected accuracy of 88%, i.e. comparable to what can be expected if homology modelling were possible for SOS (Figure 2).

How does PHD work?

The methods used to generate the automatic prediction of secondary structure by the PHD method are a profile alignment (algorithm: MaxHom/HSSP), which processes a profile of amino acid composition, and a system of neural networks that uses this profile for the prediction (PHD). The methods will be only briefly sketched here as they are described in more detail elsewhere (Schneider and Sander, 1991; Rost and Sander, 1993a).

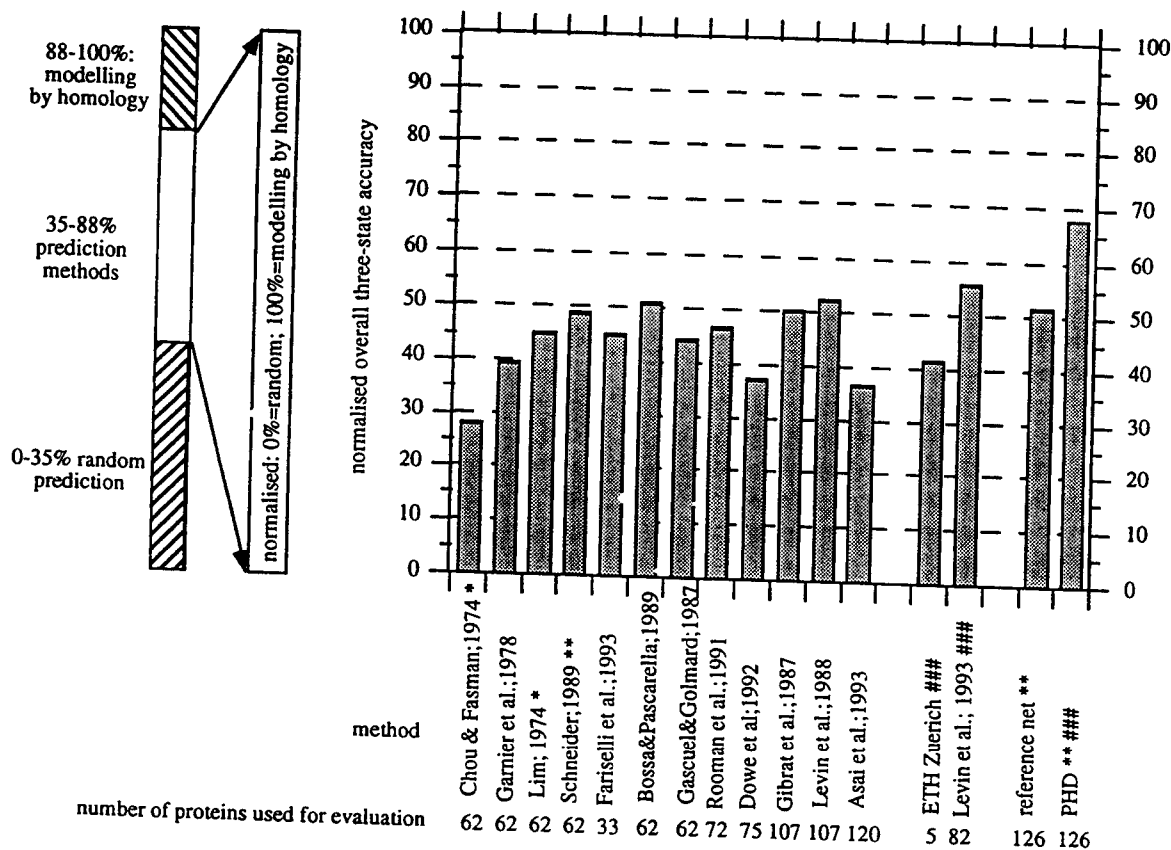


Fig. 1. Normalized overall three-state accuracy of secondary structure predictions. The values for the overall three-state (helix, strand, loop) accuracy of prediction methods are normalized such that a random prediction scores at 0%, and modelling by homology yields 100% [i.e. $Q_{3,plot} = (Q_{3,method} - Q_{3,random}) / (Q_{3,homology} - Q_{3,random})$]. Included are methods for which cross-validation has been performed without allowing pairwise sequence identity > 25% between the proteins used for evaluation. The methods are labelled according to the citation in the literature list. Those labelled with a star (*) were tested on a database of 62 proteins used by Kabsch and Sander (1983b). A double star (**) indicates results for the database of 126 proteins used in Rost and Sander (1993a). 'Reference net' describes the performance of a standard neural network (Qian and Sejnowski, 1988; Holley and Karplus, 1989) tested on 126 unique protein chains. 'PHD' labels the performance of the method used in the server. 'ETH Zurich' gives the result of the expert predictions by Benner *et al.* on five proteins (Benner and Gerloff, 1990, 1993; Benner *et al.*, 1993; Gerloff *et al.*, 1993). Levin *et al.* (1993) published a higher value of $Q_3 = 69.6\%$ for alignments of C α traces. As this figure report for using multiple sequence alignments. For both the protein set predicted by 'ETH Zurich' and 'Levin *et al.*, 1993' the PHD method scores above the average reported here (unpublished data). The methods marked with a triple hash ('###') use multiple sequence alignments as input to the prediction.

HSSP—an alignment of multiple sequences by profiles

The MaxHom/HSSP algorithm builds up the alignment in essentially two steps. In sweep 1, the sequences are aligned consecutively to the guide sequence (SOS) by a standard dynamic programming method (Smith and Waterman, 1981).

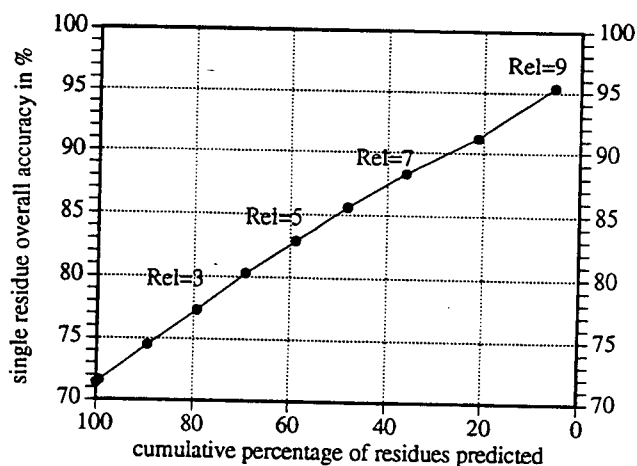


Fig. 2. Expected prediction accuracy for residues with a reliability index above a given cut-off. Plotted are averages of the three-state accuracy and the cumulative percentage of residues predicted over all those residues with reliability index (Rel) $\geq n$, $n = 0, \dots, 9$. This index is simply defined by: Rel = INTEGER $[10 * (out_{max} - out_{next})]$, where out_{max} is the output of the output unit with highest value, and out_{next} that of the unit with the next highest value. The factor 10 normalizes Rel to integer values from 0 to 9. Rel = 9 corresponds to a reliable prediction. For example, ~22% of all residues have Rel ≥ 8 and of these, 92% are correctly predicted by PHD.

After each sequence has been added to the alignment an alignment profile is compiled. This is used to align the next sequence. In sweep 2, after all sequences with significant homology have been picked from SWISSPROT, the profile is recompiled, and the dynamic programming algorithm starts once again to align consecutively the sequences, this time using the conservation profile as derived after completion of sweep 1.

PHD—a system of profile reading neural networks to predict secondary structure

The profile and the conservation weight are used as input to a first level two-layered feed-forward network ('sequence-to-structure net'). This is done by shifting a window of 13 residues successively through the sequence, i.e. the n th window starts at position n and ends at $n + 13$ of the sequence. The output of the network consists of three values between 0 and 1, which give the probability that the residue in the centre of a particular window is in a helix, strand or loop. The first level sequence-to-structure net outputs the prediction for the central residue in a window. Thus, there is no direct correlation between the secondary structure of adjacent residues. This shortcoming is corrected by feeding the output of the first level sequence-to-structure net into a second level structure-to-structure network. The architecture of the second level network is the same as for the first level network. The third level is the computation of an arithmetic average over the outputs of several independently trained two-level nets (jury decision). The networks used for the third level (jury) are trained on the same training set, the

VMS machine

- incoming mail received on a VAX computer
- pre-processing of the request:
 - assign unique job identifier
 - extract user network address from mail header and actual sequence to "job_id" file
- send job to queue and confirm request to user
- copy "job_id" file to UNIX file system (rcp) and look or wait for an idle UNIX machine
- give control to UNIX machine by starting a remote procedure call (rpc) on UNIX machine
- wait for job completion

- send result file to user or send "trouble"-mail to server operator in case of a problem.

UNIX machines

- check "job_id" file for consistency
 - correct file format?
 - is it a DNA sequence?
 - is it a BINHEX file?
- run FASTA against the latest release of the SwissProt database
- extract identifiers of homologous sequences from FASTA run
- run multiple sequence alignment program (MaxHom) against list of SwissProt identifiers and write HSSP output file
- run PHD prediction program on HSSP file
- append output files, copy result to VAX file system and exit

Fig. 3. Procedures performed by the PHD server. The Vax/VMS machine manages the incoming and outgoing mail, and sends the jobs to a cluster of four Unix machines. Here, the CPU-intensive processes are executed.

```

Joe Sequencer, Department of Advanced Protein Research,
National University, Timbuktu
joe@amino.churn.edu
# src homology-3 domain (SH3)

KELVLALYDQEKSPREVTMKKGDILLLNSTNKDWWKVEVNDRQ
GFVPAAYVKKLD
    
```

Fig. 4. Format of file to be sent to 'PredictProtein@EMBL-Heidelberg.DE'. Any format different from the one shown will result in an error message being sent back to the reader. The hash is necessary to recognize automatically that the sequence will start in the following line. If the server works fine, we do not look at the incoming prediction requests. Thus, messages to PredictProtein will remain unanswered. Instead, address queries or notes to Predict-Help@EMBL-Heidelberg.DE.

differences stem mainly from a different order of examples during the training procedure (Rost and Sander, 1993a).

PHD server—procedures between receiving and returning a mail

Communication between the VMS machine managing the incoming and outgoing mails, and Unix workstations used for the calculations is shown in Figure 3. The sequences are extracted from the mail, and the request is sent to a batch queue. From this queue, the jobs are sent to four Sun workstations (SPARC2 and 10). On the Unix side, the sequence is first checked for consistency. Then, the latest release of the SWISSPROT data bank of known sequences (Bairoch and Boeckmann, 1992) is searched for homologues. Currently, the system cuts off all sequences that have <30% sequence identity. This cut-off has been chosen as it is proven to yield true positives with high reliability. The majority of pairs in the 'twilight zone' between 25 and 30% sequence identity also have the same structure, but there also exist false positives in that region which would have to be filtered out by hand (Schneider and Sander, 1991). From the alignment the profile is computed and fed into the prediction program. Finally the result is copied (remote copy) to the VAX, and from there sent via electronic mail to the user.

How to obtain a PHD prediction

The sequence has to be written into a file according to the format shown in Figure 4. This file must be sent as electronic mail via internet to 'PredictProtein@EMBL-Heidelberg.DE'. For instructions one can send the word 'help' in the subject line to this address. Further questions, suggestions or notes should be addressed to 'Predict-help@EMBL-Heidelberg.DE'. A new option is that multiple sequence file formats (MSF; Devereux *et al.*, 1984) can be read, which means that the user can send his or her personal alignment for prediction. The alignment will then be used to compute a sequence profile in HSSP format to be used for the prediction.

```

** ALIGNMENTS 1 - 14
SeqNo PDBNo AA STRUCTURE BP1 BP2 ACC NOCC VAR
.....1.....2.....3.....4.....5.....6.....
1 6 K 0 0 184 5 24 KKK E
2 7 E - 0 0 75 12 38 EEPETTT EDDR
3 8 L E -AB 27 56A 71 12 43 LCQQLL YIIF
4 9 V E -AB 26 55A 0 12 29 VVVAFFF VVVV
5 10 L E -AB 25 54A 49 12 38 LVKRVVI RVVV
6 11 A E - B 0 53A 2 13 0 AAAAAAAAAA
7 12 L + 0 0 55 13 12 LLLLLLLLLL
8 13 Y S S- 0 0 126 13 1 YYYYYYYYFYF
9 14 D - 0 0 81 13 15 DDDDDDDDDPPD
10 15 Y B -F 20 0B 18 13 2 YYYYYYYYFYF
11 16 Q - 0 0 94 13 41 QDAEECKDDA
12 17 E + -- 0 0 61 13 27 EAAAAAGGGA
13 18 K + 0 0 149 13 46 KQERRRNIIV
14 19 S S > S- 0 0 32 13 43 SSTNTTDDHNN
15 20 P T 3 S+ 0 0 137 13 37 PPGGEEDRPPD
16 21 R T 3 S+ 0 0 158 13 36 RRDDDDSGDDR
17 22 E B < -c 47 0A 10 13 11 EEEEDDEDDDD
18 23 V - 0 0 2 13 11 VVLLLLLLLLL
19 24 T + 0 0 55 13 33 TSTTSTSPSSQ
20 25 M B -F 10 0B 3 13 21 MMFFFFFFLFFV
21 26 K > - 0 0 138 13 31 KKNTRTKKKL
22 27 K T 3 S+ 0 0 137 13 19 KKEKKKKKKK
23 28 G T 3 S+ 0 0 46 14 0 GGGGGGGGGGGG
24 29 D < - 0 0 53 14 16 DDAEEDEDEED
25 30 I E -A 5 0A 96 14 42 IVTVKRIKKKK
26 31 L E -A 4 0A 0 14 28 LLIVFFFLMLK
27 32 T E -AD 3 40A 30 14 45 TTITHQKKKKQ
28 33 L E + D 0 39A 10 15 18 LLLVVIITIVVL
29 34 L E - 0 0 59 15 22 LLLHLLLLLLLL
30 35 N E + D 0 38A 72 15 34 NNNNNNNDEERF
31 36 S + 0 0 44 15 36 SSSKNSNKEESS
32 37 T + 0 0 127 14 40 TNDSTTKPHHT.
33 38 N S S- 0 0 77 11 34 NNPNEEEgE. . .
34 39 K S S+ 0 0 164 14 48 KKKAPYGGEGGG.
35 40 D S S+ 0 0 77 14 18 DDDGDDDDGGED.
36 41 W E - E 0 49A 76 15 1 WWWMMMMMMMMWF
37 42 W E - E 0 48A 39 15 1 WWWMMMMMMMMWF
38 43 K E +DE 30 47A 63 15 40 KKKEEDERNKKG
39 44 V E -DE 28 46A 0 15 33 VVVGAAAGAAAV
40 45 E E -DE 27 45A 57 15 32 EEEERRREKKEv
41 46 V E > - E 0 44A 16 15 41 VVVLsssIDlsvv
42 47 N T 3 S- 0 0 149 15 27 NNNNstYMTsTD
43 48 D T 3 S+ 0 0 112 15 27 DDDGGGGGdKKGd
44 49 R E < - E 0 41A 108 15 35 RRRKQHGKRRRL
45 50 Q E + E 0 40A 102 15 43 QQRRRSTVREEEQ
46 51 G E - E 0 39A 2 15 8 GGGGGGGGGGGV
47 52 F E +cE 17 38A 56 15 24 FFFVYVYCMFFYF
48 53 V E - E 0 37A 0 15 17 VVVVVIIFIIIVV
49 54 P E > - E 0 36A 16 15 0 PPPPPPPPPPPPP
50 55 A G > S+ 0 0 15 15 32 AAAAASSVSSSP
51 56 A G 3 S+ 0 0 76 15 34 AAANSNNNNPNA
52 57 Y G < S- 0 0 89 15 1 YYYYYYYYFYF
53 58 V E < -B 6 0A 11 15 10 VIVVVVVVVVVC
54 59 K E -B 5 0A 107 15 39 KKRQEAEEAAEA
55 60 K E -B 4 0A 82 15 43 KKRDLPPPKKPG
56 61 L E B 3 0A 64 13 16 LLIIIVV LVVV
57 62 D 0 0 193 12 29 DDD PDDD NNEA
    
```

Fig. 5. Returned sequence alignment in HSSP format. Example for the SH3 protein (src homology region 3). The format of the multiple alignment is the same as the one used in the HSSP database of protein structure-sequence alignments. For the current PDB-SWISSPROT version the HSSP files are available via anonymous ftp from ftp.EMBL-Heidelberg.DE (Schneider and Sander, 1993). Abbreviations: SeqNo, numbering from first to last residue; PDBNo, position numbers from the related PDB file (column empty if there is no PDB file); AA, one-letter code for residue; STRUCTURE, secondary structure assignment according to DSSP (if three-dimensional structure is unknown this column gives a 'U' for unknown, not the predicted secondary structure); BP1 and BP2, positions of bridge partners in β -sheets; ACC, solvent accessibility as calculated by the DSSP program (Kabsch and Sander, 1983a); NOCC, number of sequences which are aligned at that position; VAR, residue type variability at that position. Lower-case characters in the alignment indicate deletions and dots mark insertions.

Soon after the sequence has been sent to the server, a message is sent to the user, confirming that the job has been sent to the queue. After the job has been processed, the user ought to receive the mail containing the output of the multiple alignment generated by the program MaxHom (Schneider and Sander, 1991) and written in the format of the HSSP files (Figure 5),

```

PHD output for your protein:
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Abbreviations:
-----
secondary structure : H=helix, E=extended (sheet), blank or L=rest (loop)
                    AA: amino acid sequence
                    PHD: Profile network prediction HeiDelberg
                    Rel: Reliability index of prediction (0-9)
detail:
    prH: 'probability' for assigning helix      --
    prE: 'probability' for assigning strand
    prL: 'probability' for assigning loop
    note: the 'probabilites' are scaled to the interval 0-9,
          prH=5 means, that the signal at the first output no
          is 0.5-0.6.
subset:
    SUB: a subset of the prediction, for all residues with
          an expected accuracy > 82% (see tables in header)
    note: for this subset the following symbols are used:
          L: is loop (for which above " " is used)
          ".": means that no prediction is made for this residue,
              as Rel < 5

          . . . . .1 . . . . .2 . . . . .3 . . . . .4 . . . . .5 . . . . .6
AA |KELVLALYDQEKSPREVTMKKGDILLLNSTNKDWWKVEVNDRQGFVPAAYVKKLD|
Obs | EEEE E E EEEEE EEEEE EEEEEHHEEEEE |
PHD | EEEEEEE EE EEEEE EEE EEE EEE |
Rel |946888762246799763121574799971787321124368984221311133169
detail:
prH-|000000001221000111011101000001001233332211000011134311100
prE-|037888774211100013453212788874101122455210003554212455420
prL-|862101123456788775434676100014787543111567886334543223478
subset: SUB |L.EEEEE...LLLLL...LL.EEEEE.LLL.....LLLL.....LL|

```

Fig. 6. Returned prediction of the PHD method. Example for the SH3 protein (src homology region 3). The PHD prediction is summarized in three lines: AA, echo of the sequence of SH3; PHD, prediction in three states helix (H), strand (E) and loop (blank); and Rel, the reliability index from 0 to 9, indicating a prediction site of highest reliability (definition as in Figure 2). Note: for comparison the DSSP (Kabsch and Sander, 1983a) assignment of the three-dimensional structure for SH3 (Musacchio *et al.*, 1992) is given (Obs). The second block of three lines dubbed 'detail' reports the probability of the assignment: the system of neural networks has three output units for helix, strand and loop, which can adopt values between 0 and 1. In the usual prediction (row 3: PHD) the highest of these units is chosen as the prediction. The detail gives the actual value for each unit (projected onto a grid from 0 to 9). This quantity supplies a probability for the assignment of helix, strand and loop. The last row (subset: SUB) repeats, for a quick overview, the same assignment as given in the third row (PHD), except that now only those assignments are given for which the prediction has an unexpected reliability index ≥ 5 . For this subset the expected reliability is $> 82\%$ (Figure 5).

and of the prediction of secondary structure in three states (Figure 6). Should > 3 days elapse between the confirmation and the arrival of the prediction, we ask the user to write a note to Predict-Help@EMBL-Heidelberg.DE.

Recommendations after more than 4000 predictions

By September 15, 1993 > 5500 predictions have been requested, and > 4500 have been made (Figure 7). For ~ 1000 sequences no homologues were found in the sequence database. The number of requests varies considerably from one country to another and is obviously affected by the size of the country, the level of activity in computational molecular biology, and the availability of molecular biology network services (Figure 8). For future requests, the user should keep the following points in mind.

Use homology prediction whenever possible

On occasion, the server alignment search turns up a homology to a protein of known three-dimensional structure. This is apparent from the presence of a four-letter PDB identifier (e.g.

5MBN for myoglobin) in the list of homologous proteins returned. In these cases, the predicted secondary structure gives, by definition, much less information than a three-dimensional model built using standard homology modelling tools.

Patterns not used for training cannot be predicted

The training of the network system was done on particular examples of native and dominantly globular proteins with 13 consecutive residues on the first level and 17 consecutive residues on the second level. Thus, the predictions cannot be expected to yield a comparable accuracy for:

- membrane proteins: average overall accuracy in three states was as low as 56% for porin (3por), melittin (2mlt), and the trans-membrane segments of the photo-reaction centre (1prc);
- fragments of < 13 consecutive residues;
- differences between wild-type and point mutants;
- fragments containing a considerable percentage of unknown amino acids.

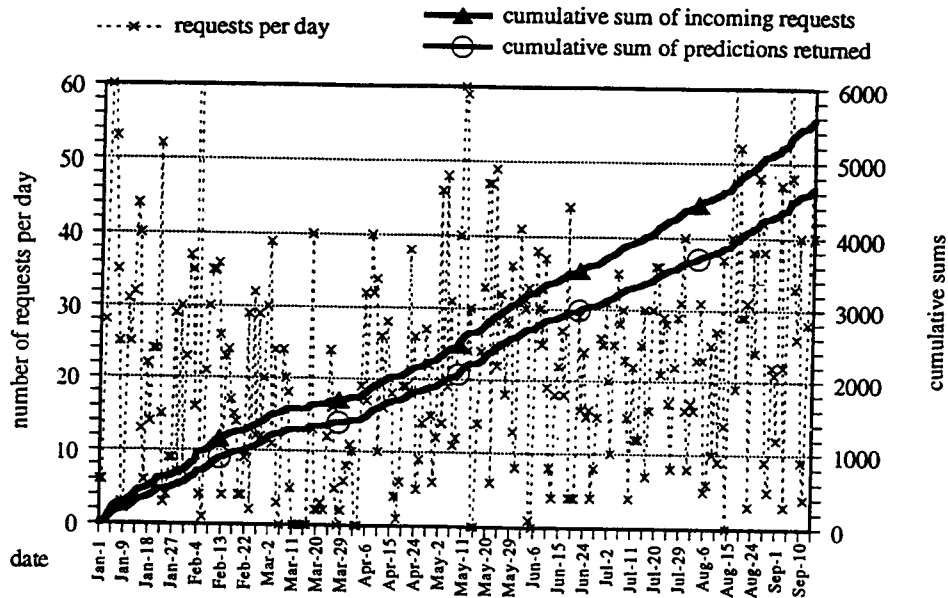


Fig. 7. Number of prediction requests from January to September 15, 1993. The difference between the requests and the number of predictions sent is partly explained by the fact that for the first four months no prediction was returned if no homologue to the sequence sent had been found in the current release of SWISSPROT. The decision to send predictions only if there was at least one homologue was motivated by the fact that only for this case has the network system been proven to be significantly better than alternative predictions. Meanwhile, we have lifted that restriction, as there is no evidence that the network without multiple alignment information is significantly worse than other prediction methods available [and is definitely much better than the Chou–Fasman method (Chou and Fasman, 1974)]. Expected accuracy figures, however, are for cases in which homologues are available. The server has become rather complex. This caused many difficulties, like the machine crash in the middle of March that led to a backlog of the requests.

The prediction depends on the quality of the multiple alignment

The accuracy of the prediction depends crucially on the information contained in the multiple alignment. PHD is significantly better than previously published methods only if a reasonable multiple sequence alignment can be made. Not only is the number of homologues important, but also the diversity of the family. It is better, in most cases, to have 50 sequences with 30–90% sequence identity relative to the guide sequence, than to have 100 sequences all in the range of 70–90% identity.

The prediction of the network, as given by the symbols H, E and L, can be rather sensitive to changes in the details of an alignment. However, this sensitivity does not hold for regions that are predicted with a high reliability index (Figures 2 and 6). Consequently, the prediction returned might be different for a different release of SWISSPROT for a given version of the trained networks. The probabilities for helix, strand and loop as given in the output of the returned prediction change only marginally for small deviations in the alignment profiles.

Cut-off in sequence similarity for the compilation of the multiple alignment

There is a length-dependent cut-off for structural similarity as a function of sequence similarity (Schneider and Sander, 1991). However, the cut-off is not razor sharp. Instead, there is a 'twilight zone' of, say, 3 percentage points (in sequence identity) above the cut-off line. The publicly available HSSP data bank

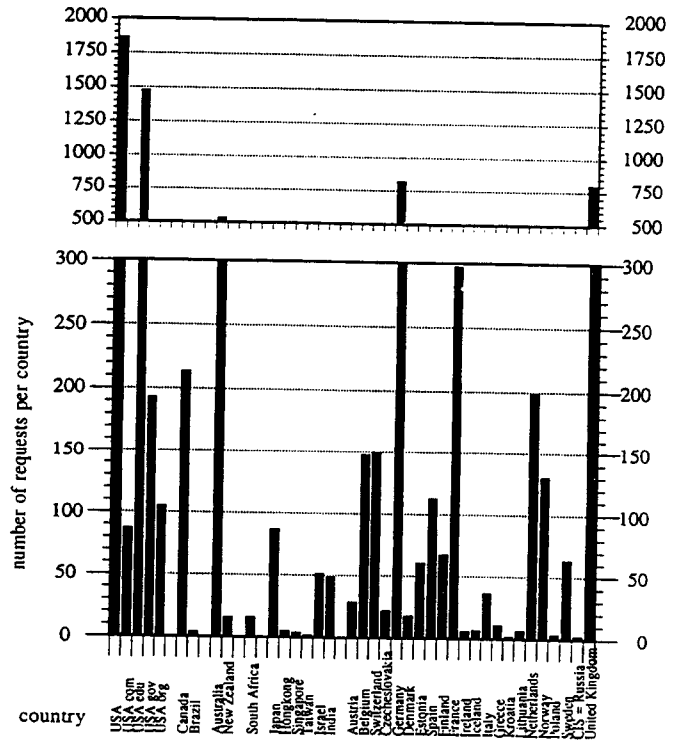


Fig. 8. Number of prediction requests per country. The level of server requests in a particular country reflects the number of molecular biology laboratories, the level of activity in computational molecular biology, the availability of network services or a combination of these and other factors.

uses the safety margin of 'cut-off + 5 percentage points', i.e. identical residues for alignments of length >80. Experiences with a set of 124 recently solved structures (B.Rost and C.Sander, unpublished results) have shown that the prediction accuracy is improved if the threshold is lowered to the threshold for structural homology defined earlier (Schneider and Sander, 1991). However, this may on occasion include spurious alignments, i.e. alignments with structurally non-similar proteins. Therefore, we currently use the safety margin of plus 5 percentage points for the server.

No consensus prediction

Although the network system uses the information contained in a sequence family, each prediction is derived for the secondary structure of the *guide* sequence. Strictly speaking, therefore, the result is not a consensus prediction for a family. A simple, practical way for every user to derive a consensus prediction is to predict the secondary structure for each sequence in a family, to bring the sequences in frame and then to sum up the probabilities for each output unit helix, strand and loop (given in the prediction output) at each alignment position over all predictions (in the sum, a weight reflecting the rarity of the sequence in the family should in principle be used). This is different from merely counting the predicted symbols at each position, an approach that would be inconsistent with our basic method. The last step is to assign the consensus prediction at each alignment position to the unit (helix, strand, loop) with the maximal sum. In practice, however, the difference between the prediction for the guide sequence and the consensus prediction for the entire family is marginal, so in most cases the server prediction can be safely taken as the family *consensus* prediction.

Uniqueness of the prediction

Since the service started, we have on three occasions changed the architecture of the networks used. The method is in the process of being improved further. Therefore, predictions sent at different times may differ not only as a result of database updates, but also as a result of updates in the method (see version number).

Conclusion

Over the last 20 years, some 100 methods for the prediction of protein secondary structure have been published. Only some of these methods are available to biologists who actually need the prediction tools. The PHD server gives access to a method that predicts secondary structure of globular proteins with an expected accuracy of 71.4%, if at least one homologue sequence can be aligned. The overall accuracy is some 5 percentage points better than any other method published (Zhang *et al.*, 1992). About 40% of all residues have an expected accuracy comparable to that achieved by homology modelling, i.e. modelling based on homology to a known three-dimensional structure. In comparison, for GORIII (Gibrat *et al.*, 1987) this

value is reached for ~15% of all residues and for an alternative earlier neural network (Holley and Karplus, 1989) for ~10% of all residues. All the potential user needs is an electronic mail connection. This makes the service available also to users working in smaller laboratories without access to significant computer power. The speed of the prediction is sufficient to keep up with the amount of data produced by large-scale sequencing projects.

Acknowledgements

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