PROFtmb: a web server for predicting bacterial transmembrane beta barrel proteins

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Received February 14, 2006; Revised March 1, 2006; Accepted March 31, 2006

ABSTRACT

PROFtmb predicts transmembrane beta-barrel (TMB) proteins in Gram-negative bacteria. For each query protein, PROFtmb provides both a Z-value indicating that the protein actually contains a membrane barrel, and a four-state per-residue labeling of upward- and downward-facing strands, periplasmic hairpins and extracellular loops. While most users submit individual proteins known to contain TMBs, some groups submit entire proteomes to screen for potential TMBs. Response time is about 4 min for a 500-residue protein. PROFtmb is a profile-based Hidden Markov Model (HMM) with an architecture mirroring the structure of TMBs. The per-residue accuracy on the 8-fold cross-validated testing set is 86% while whole-protein discrimination accuracy was 70 at 60% coverage. The PROFtmb web server includes all source code, training data and whole-proteome predictions from 78 Gram-negative bacterial genomes and is available freely and without registration at http://rostlab.org/services/proftmb.

INTRODUCTION

Transmembrane beta-barrel (TMB) proteins form a beta-barrel as a single beta-sheet joined at its edges. The sheet is ‘all-next-neighbor’, meaning all paired strands are adjacent in sequence. N- and C-termini of TMBs always reside in the periplasm. The architecture can be described as the repeating sequence, an email of the results URL is sent. PROFtmb, originally published in (2) provides a four-state (upward-strand, downward-strand, outer loop, periplasmic loop) per-residue prediction. Graphical output consists of color-coded four state posterior probability plots and amino acid sequence (Figure 1). Amino acid color indicates the final prediction, and usually corresponds to the state with maximum posterior probability, but with ‘corrections’ based on context shown with lighter-weight font [described in ‘Decoding’ section of the Supplementary Data of (2)]. While we did not quantify confidence levels for per-residue prediction, higher Z-values tend to have fewer corrected residues and greater contrast in state posterior probabilities.

In the example shown (Figure 1), OMPA from Escherichia coli [PDB: 1g90 (5) chain A] is predicted correctly at high confidence as an eight-stranded TMB. This result is expected, given PROFtmb was trained on very similar sequences. In most predictions on real TMBs, corrected residues are only...
found at the boundaries between strands and loops. Also, most strand and loop states have the best state close to probability 1.

In the second example shown (Figure 2), heme acquisition system protein A from *Serratia marcescens*, of the gammaproteobacteria class (Gram-negative) illustrates a false positive prediction. It receives a low but above-threshold Z-value of 4.8. In fact, the structure [PDB: 1B2V (6)] consists of a seven-stranded beta-sheet against four \( \alpha \)-helices. PROFtmb does correctly predict the locations of five of the strands. Notice that predicted strands four, five and six have poor contrast in posterior probability, indicating a poor fit to the PROFtmb model.

Finally, proteins shorter than 140 or longer than 1392 residues receive Z-value \(-10\,000\) (data not shown). The lower length of 140 is a conservative estimate of the smallest possible TMB, while the upper bound reflects the limit of our test set for Z-value calibration.

Occasionally, PROFtmb will assign Z-value less than four to a known TMB. Unfortunately, in such a case, the fact that it is a TMB can’t be used to help produce a reliable per-residue prediction since PROFtmb derives the prediction from sequence alone. This occurred in about 15% of the cases in our test set (see ‘Performance Evaluation’ in ‘Methods’ tab on website).

**DISCUSSION**

In our original paper (2) we used PSI-BLAST profiles run with options \(-h 1\) (E-value cutoff for inclusion in next pass) and \(-j 2\) (number of iterations), and did not explore the effect of different profiles on PROFtmb accuracy, either for whole protein or per-residue prediction. Since then, we have run 8-fold jackknife tests (leave one out, seven in) on the original SWISS-PROT sequence versions of eight PDB structures (SetTMBfull: 1a0s_P, 1af6_A, 1bt9_A, 1fep_A, 1lpr, 1qd5_A, 1q9_A, 1qj9_A). We built sets of PSI-BLAST profiles with 30 different combinations of settings \(-h \{1,0.1,0.01,0.001,0.0001,0.00001\}\) and \(-j \{2,3,4,5,6\}\) and used each set in a separate jackknifed test. The original Q2 accuracy, with settings \(-h 1 \rightarrow j 2\) was 86.0%, while the best settings, \(-h 0.0001 \rightarrow j 2\) achieved 87.3% Q2 accuracy. As a result, we changed the defaults to \(-h 0.0001 \rightarrow j 2\). Additionally, we now allow the user to select these parameters. We have not estimated the effects of PSI-BLAST settings on whole-protein prediction yet. Currently, Z-value and resulting estimated accuracy and coverage are calibrated from our
original sequence-unique set called SetROC, containing a representa-
tive set of proteins from SWISS-PROT. As sequence
databases are updated, we will periodically re-calibrate
Z-values. A cluster plot and resulting accuracy versus coverage
curve can be found in the ‘Methods’ section of the website.

DOWNLOADS

Predictions on 78 Gram-negative proteomes are available in the
Download section, updated since original publication as fol-
lows. First, length-adjusted bits score was replaced by
Z-value, which gives slightly improved discrimination on our test set
(unpublished data). Second, per-residue predictions were re-run
using updated PSI-BLAST profiles, with option /C0
rather than /C0
Both changes are expected improvements,
but haven’t been rigorously tested. Third, the model archite-
cture now explicitly includes BEGIN and END states, represent-
ing the beginning and end of the amino acid sequence. This is
required for the current version of the software.

The PROFtmb software is a general profile-HMM allowing
specification of model architecture, encoding and decoding.
The training data, consisting of eight TMB sequences
with hand-annotated per-residue labeling based on their
3D structures, is available as well. Interested users may down-
load and compile the C++ source code and use PROFtmb
with the original training data or modify it. We make it available in
the spirit of reproducibility, and encourage interested readers to
contact the authors for more detailed advice.

ACKNOWLEDGEMENTS

Thanks to Pier Luigi Martelli and Pantelis Bagos for helpful
discussions, generous use of data and sharing unpublished
ideas. Thanks to Amos Bairoch (SIB, Geneva), Rolf Apweiler
(EBI, Hinxton), Phil Bourne (San Diego University), and their
crews for maintaining excellent databases and to all experi-
mentals who enabled this analysis by making their data public-
ly available. Last, not least, thanks to all those who deposit
their experimental data in public databases, and to others who
maintain these databases. This work was supported by grant
R01-LM07329-01 from the National Library of Medicine.
Funding to pay the Open Access publication charges for this
article was provided by NIH/NLM R01-LM07329-01.

Conflict of interest statement. None declared.

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