FOR THE RECORD

MPEx: A tool for exploring membrane proteins

Craig Snider,[1] Sajith Jayasinghe,[2] Kalina Hristova,[3] and Stephen H. White[1]*

[1]Department of Physiology and Biophysics, Center for Biomembrane Systems, University of California, Irvine, California 92697-4560

[2]Department of Chemistry and Biochemistry, California State University, San Marcos, San Marcos, California 92096-0001

[3]Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, Maryland 21218

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Abstract: Hydropathy plot methods form a cornerstone of membrane protein research, especially in the early stages of biochemical and structural characterization. Membrane Protein Explorer (MPEx), described in this article, is a refined and versatile hydropathy-plot software tool for analyzing membrane protein sequences. MPEx is highly interactive and facilitates the characterization and identification of favorable protein transmembrane regions using experiment-based physical and biological hydrophobicity scales. Besides allowing the consequences of sequence mutations to be examined, it provides tools for aiding the design of membrane-active peptides. MPEx is freely available as a Java Web Start application from our web site at http://blanco.biomol.uci.edu/mpex.

Keywords: topology; structure prediction; transmembrane helices; hydropathy plots; hydrophobicity; membrane protein explorer

Introduction

Elucidating the three-dimensional structures of membrane proteins continues to be a demanding and often slow process. Despite the exponential increase in the number of unique structures,[1,2] many years will be required to obtain a representative collection of structural motifs.[3] Biochemical and molecular biological studies guided by structure-analysis software is likely to be an important approach to membrane protein structure determination for many years to come, especially because topology prediction has improved significantly in the past few years.[4]

Sliding-window analysis of sequence hydrophobicity is a simple method for identifying putative TM segments of membrane protein sequences. Popularized by Kyte and Doolittle,[5] sliding-window hydropathy analysis is often the first step in the analysis of secondary structure. A number of sophisticated software tools descended from the Kyte–Doolittle work can be found at http://ca.expasy.org/tools/. Although these tools are extremely useful, we designed membrane protein explorer (MPEx) to aid specific research projects of our laboratories. The first version of MPEx was born to aid our studies of the role of peptide bonds in the energetics of transmembrane helix stability,[6] but evolved over time to serve other research needs, such as studies of the relative contribution of hydrophobic

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*Correspondence to: Stephen H. White, Department of Physiology and Biophysics, Center for Biomembrane Systems, University of California, Irvine, CA 92697-4560. E-mail: stephen.white@uci.edu

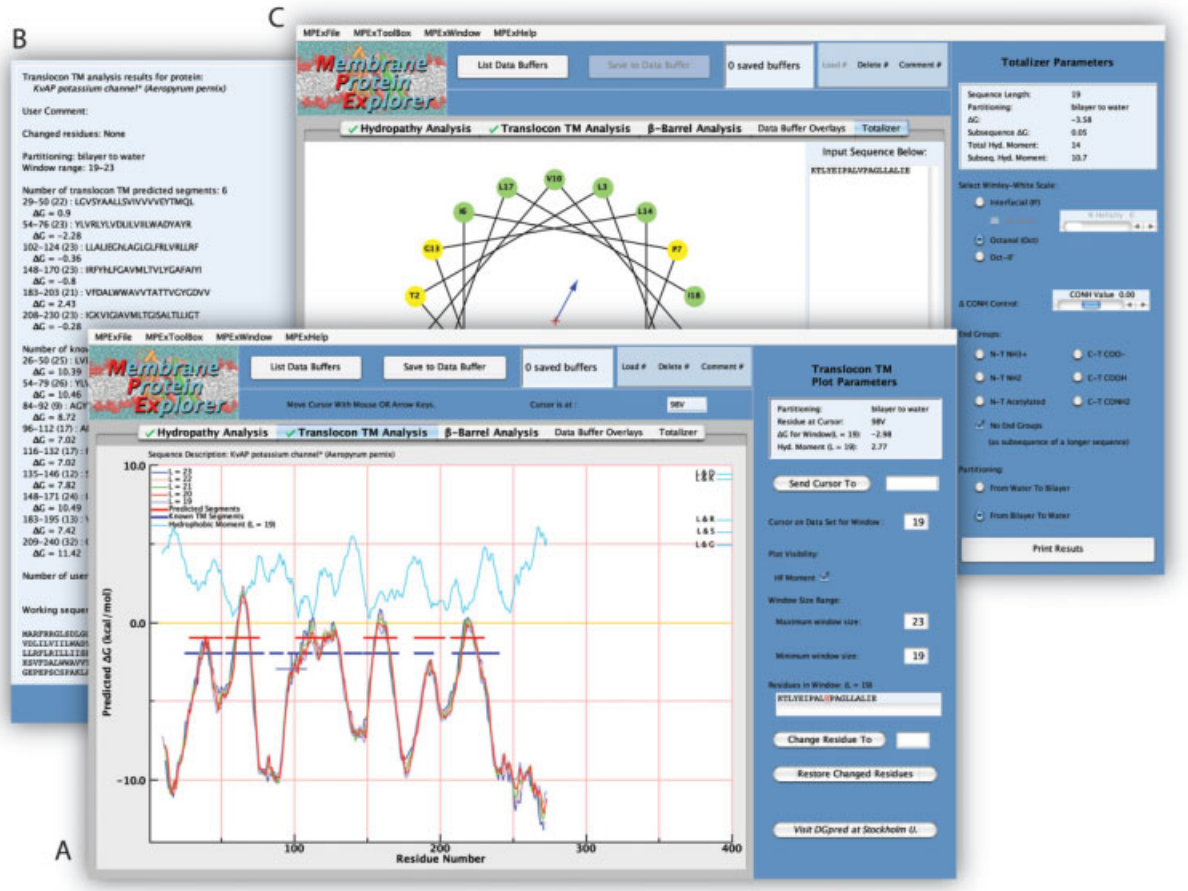

Figure 1. MPEx screen shots. (A) MPEx in translocon TM analysis mode. The window consists of three main sections: The top portion provides standard application type pull-down menus for operations like I/O or functions common across different analysis modes, data buffer controls, and tabs for analysis mode selection. The white region to the lower left displays analysis plots, and the shaded region to the right contains the data summary window and control panel. The control panel allows selecting the plotting region cursor location, the sliding window size range, and changing or restoring a changed residue in the current analysis sequence. (B) Results window for translocon TM analysis, displaying control settings, analysis results, and working amino acid sequence. (C) MPEx in totalizer mode. Totalizer mode is primarily for the analysis of short amino acid sequences and the design of membrane-active peptides. Totalizer generates a helical wheel display for the sequence being examined, along with an arrow indicating the direction and relative magnitude of the hydrophobic moment for the sequence.

moment to translocon recognition of transmembrane segments[7] and peptide partitioning into membrane interfaces.[8] MPEx is a now a highly refined implementation of the sliding-window method that utilizes primarily experiment-derived physical[6] and biological[9] hydrophobicity scales, although other scales can be used. Implemented in Java (http://www.java.com), MPEx is a tool that provides an extendable framework not only for physical and biological hydropathy analyses, but β-barrel identification screening as well. MPEx also calculates and displays hydrophobic moment data, generates helical wheel displays, and allows direct comparison of different sliding-window plots.

General Features of MPEx

The graphical user interface of MPEx is divided into a plot window and a control panel, used for setting the plot parameters for the analysis mode [Fig. 1(A)]. MPEx has three analysis modes of operation and two utility modes that are available from a convenient tabbed interface. The analysis modes include physical-scale hydropathy analysis,[6] translocon-scale hydropathy analysis,[9] and β-barrel analysis.[10] The utility modes include Totalizer for estimating the binding free energies of peptides to phosphatidylcholine interfaces.[11] The other utility mode is Data-Buffer Overlay, which allows storage and graphical comparisons of different sliding-window plots. With this feature, for example, one can compare hydropathy plots of different proteins, or the same protein with amino acid mutations.

MPEx users can enter sequences for analysis in several ways: Sequences may be typed directly into MPEx, read from a saved sequence file, or read directly from the SwissProt[12] or MPtopo[13] databases. Results generated by MPEx include the analysis-plot data as well as a text output listing the TM segments predicted by the program and the parameter settings

used to derive the generated data [Fig. 1(B)]. Moving the cursor in the plot window along the analysis plot displays the residues included in the sliding window in the "Residues in Window" box on the control panel. The center residue in the window is highlighted in red. The MPEx plot window can be printed directly (optionally including the control panel), or plot data can be saved in ASCII format suitable for importing into spreadsheet programs such as Microsoft Excel or Microcal Origin, which makes it easy to produce publication-quality plots. Graphics and text generated by MPEx can be printed or, in the case of the text output, saved to disk. Work sessions may be saved and restored between invocations of MPEx.

Hydropathy Analysis Mode

In hydropathy analysis mode, hydropathy plots are generated using the augmented Wimley–White (WW) experiment-based whole-residue hydrophobicity scale described by Jayasinghe et al.[6] This augmented scale relies on measurements of the partitioning of hydrophobic pentapeptides[14,15] and salt-bridge pairs[16] into *n*-octanol. Unlike other hydrophobicity scales, these account for the energetic cost of partitioning the peptide backbone as well as the side chains. Hence, they are whole-residue scales. Jayasinghe et al.[6] have shown that accounting for the high-energetic penalty of peptide-bond partitioning is crucial for accurate detection of transmembrane helices in membrane protein sequences. Wimley and White[17] also measured the energetics of partitioning whole amino acid residues into the interface of phosphatidylcholine membranes, leading to a whole-residue interfacial (IF) hydrophobicity scale. Users of MPEx may choose the WW octanol scale or the WW IF scale for hydropathy plots. In addition, users may choose to make hydropathy plots using the free energy differences between the WW octanol and IF scales (Octanol-IF scale), which estimates the relative preference of an amino acid sequence for partitioning as an unfolded chain into a phosphatidylcholine membrane interface or as an α-helix across the membrane. The free energy differences between the protonated and deprotonated forms of Asp, Glu, and His are included in both the octanol and IF scales. This makes it possible to determine the effects of the protonation states of these residues on hydropathy plot results.

The sliding-window size for the hydropathy plots is 19 AA in the locate mode or freely selectable in the scan mode. The locate mode is distinguished from the scan mode by inclusion of the algorithms described by Jayasinghe et al.[6] for identifying candidate transmembrane helices. The octanol scale is the default scale for hydropathy analysis.

Translocon TM Analysis Mode

Translocon TM analysis mode is a hydropathy analysis of transmembrane proteins based on the molecular code for transmembrane-helix recognition by the Sec61 translocon.[9,18] This code not only provided measurements of the apparent hydrophobicity scale used by the translocon, it established that the apparent hydrophobicity value of a residue depends on its location within the TM segment. For example, the hydrophobicity of Trp is very favorable when it is located at the ends of TM helices but is much less favorable when located in the center of a TM segment. Such position dependences and helix length are accounted for in the molecular code. An algorithm for identification of TM segments using the molecular code has been implemented[9] as ΔGpred, a server-based analysis tool available at http://www.cbr.su.se/DGpred. This ΔGpred algorithm is used for the translocon TM analysis mode in MPEx. As in ΔGpred, users may select a range of sliding window sizes, and plots are calculated for each of them. Predictions of transmembrane regions are based on comparison of favorable regions among the different window sizes, with the most favorable among conflicting or overlapping regions being selected [Fig. 1(A)].

β-Barrel Analysis Mode

The β-barrel analysis mode does a screening analysis for the identification of β-barrel membrane proteins based on the analysis algorithm of Wimley.[10] The algorithm uses amino acid composition and architecture of β-barrel proteins of known structure to make predictions of TM β-strands, connecting β-hairpin loops, and the number of likely TM β-strands. Scores are assigned for the likelihood of an entire analysis sequence representing a β-barrel motif membrane, the number of β-strand peaks, and for windows of user selected sizes representing two potential β-strand regions and the connecting β-hairpin loop. Predictions of β-strand and β-hairpin regions are made based on these scores.

Utility Modes

MPEx provides a utility analysis module, Totalizer [Fig. 1(C)], that is designed for investigating short amino acid sequences such as peptides or protein segments and should be particularly useful in the design of membrane-active peptides. Totalizer calculates water-to-bilayer or bilayer-to-water transfer free energies of short sequences using the Wimley–White hydrophobicity scales. In addition, Totalizer provides the ability to investigate the consequence of acetylating the N-terminus or amidating the C-terminus on bilayer partitioning using free energy values and an algorithm determined by Hristova and White.[11] MPEx also provides the ability to determine transfer free energies of sequences to the bilayer interface as a function of α-helical content based on measurements of Ladokhin and White.[19] Future versions of Totalizer will account for electrostatic interactions of peptides with bilayer interfaces[20] and for the strong effects of peptide helical hydrophobic moments.[8] A particularly

useful feature of Totalizer is helical-wheel plots of peptide hydrophobic moments [Fig. 1(C)].

The MPEx Data Buffer utility allows saving and restoring analysis data and parameter settings for protein sequences to and from data buffers. The data sets stored in the data buffers can be directly compared with one another using the Data Buffer Overlays utility mode that superimposes hydropathy plots of different proteins or proteins with modified sequences.

Novel Features of MPEx

One of the most common ways of studying membrane protein function is by site-directed mutagenesis. How might a particular mutation affect the TM stability of a putative TM helix? This question is easily investigated in MPEx using the "change residue to" box. The plot-window cursor is moved along the sequence until the residue of interest is highlighted in red. The replacement residue is entered into the "change residue to" box and the control button pressed. MPEx tracks such sequence changes, allowing all changed residues to be reverted to the original ones.

Because the WW hydrophobicity scales include values for the deprotonated and protonated versions of Glu, Asp, and His, MPEx makes it easy to change the charge state of these residues by pushing the "change charge" button on the control panel. MPEx assumes a pH of 7.0, meaning that the defaults are negatively charged Asp and Glu and neutral His. The WW scales also include values for salt-bridged pairs. The "set salt bridges" button on the control panel allows charged residues in the sequence to be salt-bridged to examine the consequences of salt bridging in the physical-scale hydropathy plots. The effects of charge state and salt bridges in the translocon molecular code are unknown; consequently, the change-charge and salt-bridge options are not available in the translocon TM analysis mode.

Statistical studies of known membrane proteins as well as experimental evidence suggest that charged and aromatic residues play important roles in determining TM segment topology.[21–23] Generally, the preferential positioning of amino acids seems to play an important role in membrane-protein assembly.[7,9] Determining the location of residues such as arginine, lysine, and tryptophan in hydropathy plots helps identify segment ends in topology prediction. MPEx provides the ability to display the locations of specific amino acid types in the analysis plots. This feature can be used, for example, to locate positively charged residues in the context of the "positive inside rule"[24] or to locate aromatic residues.

Finally, MPEx always computes, in the hydropathy and translocon TM analysis modes, the helical hydrophobic moments of the sliding windows using the selected scale. The HF moment sliding-window plots are not visible by default; they can be made visible from the control panel. Hydrophobic moments are useful for identifying regions of sequences that may have a high propensity for the membrane interface.

Availability

MPEx and its complete documentation are freely available at http://blanco.biomol.uci.edu/mpex. Visiting the web site and clicking on the "Start MPEx" button will guide the user through the acquisition of the Java virtual machine and Web Start technology required for installing MPEx. The Java/Web Start technology acquisition step will be bypassed if it is already present on the user's computer. A valuable feature of the Web Start technology is that the parent web site is checked each time a Web Start program is started. If a newer version of the program is available, the software will be automatically updated.

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