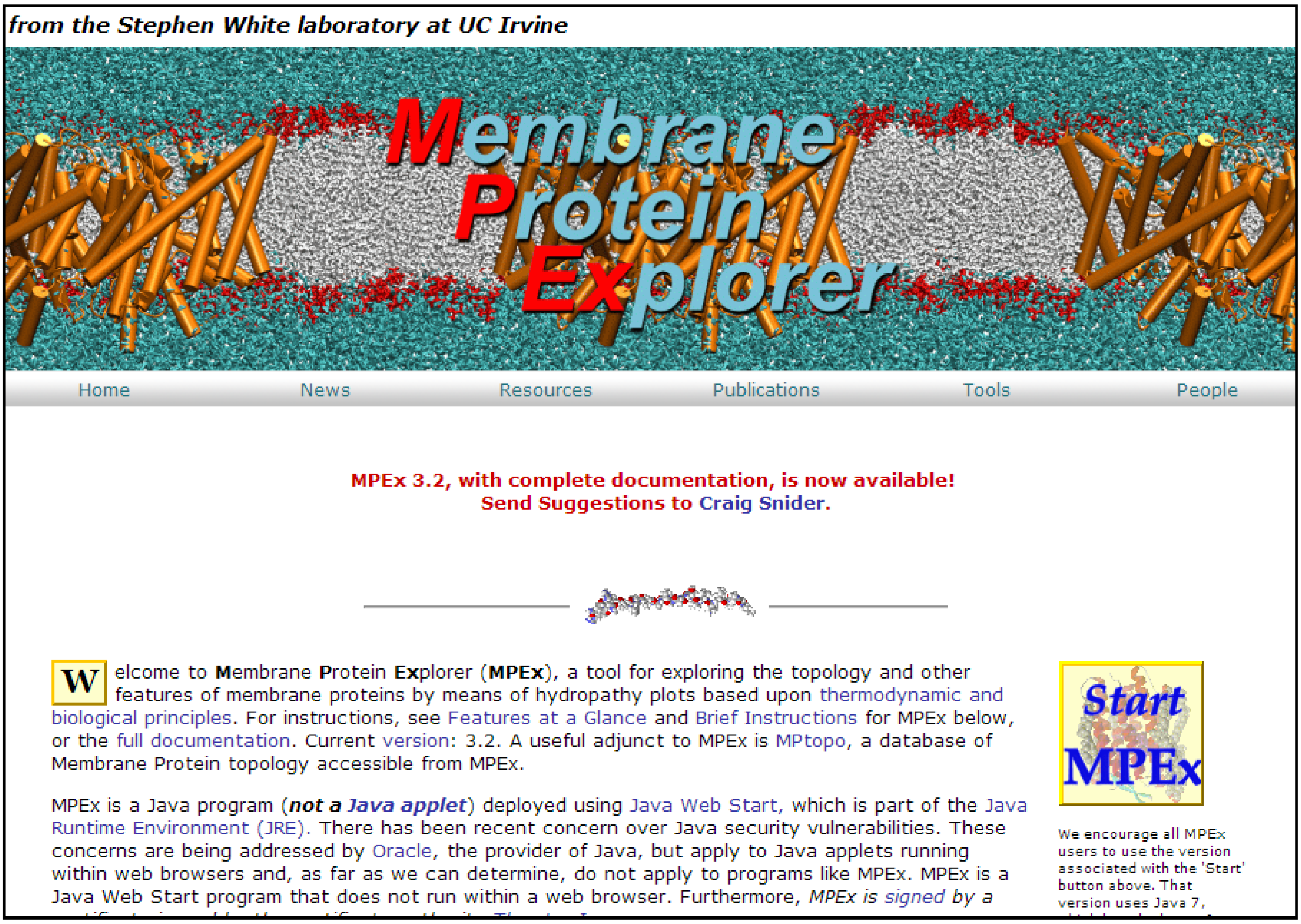
Supplemental Information: *Peptide entry inhibitors of enveloped viruses: The importance of interfacial hydrophobicity.*

H. Badani, RF Garry, WC Wimley

Using MPEx: Membrane Protein Explorer



from the Stephen White laboratory at UC Irvine

MPEx 3.2, with complete documentation, is now available!
Send Suggestions to Craig Snider.

Welcome to **M**embrane **P**rotein **Ex**plorer (**MPEx**), a tool for exploring the topology and other features of membrane proteins by means of hydropathy plots based upon thermodynamic and biological principles. For instructions, see Features at a Glance and Brief Instructions for MPEx below, or the full documentation. Current version: 3.2. A useful adjunct to MPEx is MPtopo, a database of Membrane Protein topology accessible from MPEx.

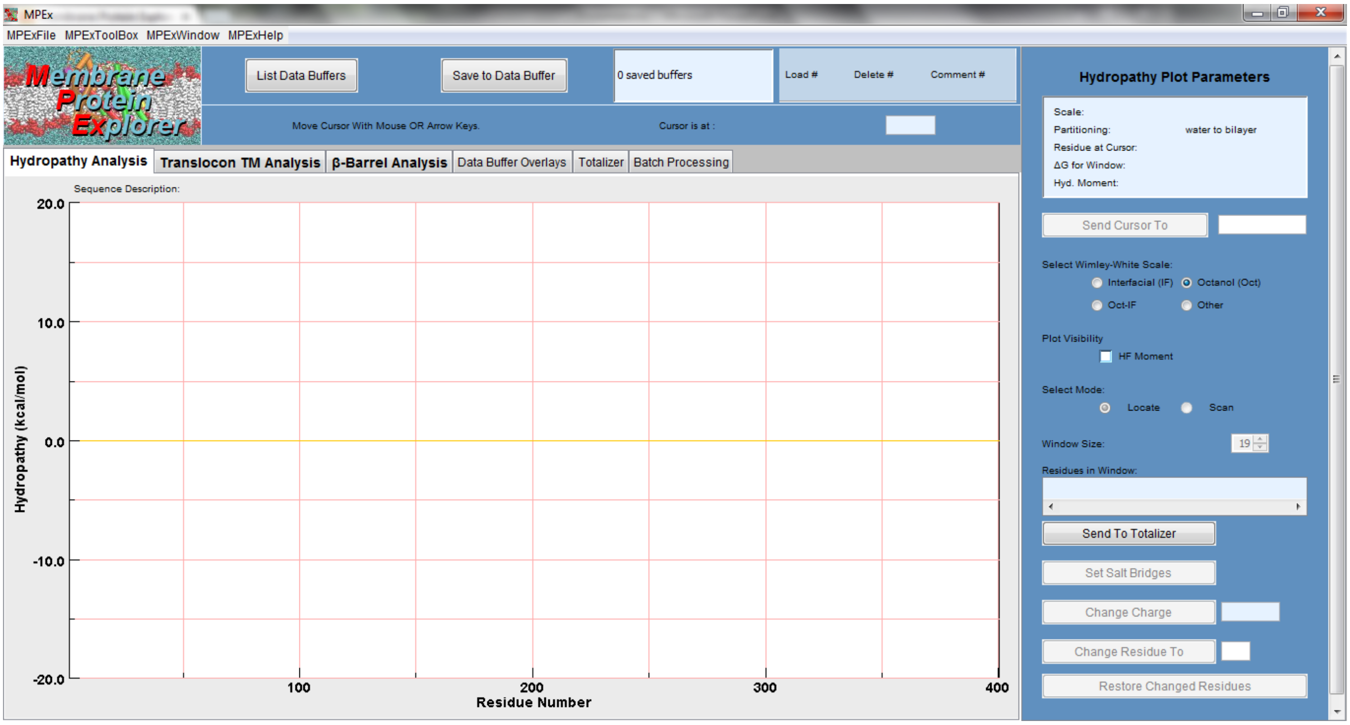
MPEx is a Java program (***not a Java applet***) deployed using Java Web Start, which is part of the Java Runtime Environment (JRE). There has been recent concern over Java security vulnerabilities. These concerns are being addressed by Oracle, the provider of Java, but apply to Java applets running within web browsers and, as far as we can determine, do not apply to programs like MPEx. MPEx is a Java Web Start program that does not run within a web browser. Furthermore, *MPEx is signed by a*

We encourage all MPEx users to use the version associated with the 'Start' button above. That version uses Java 7,

1) Navigate your browser to: http://blanco.biomol.uci.edu/mpex/

2) Click “Start MPEx”. A java program (certified to be safe) will be deployed on your computer. Note its location or make a shortcut to it and you can run it from there in the future without needing an internet connection. You must have java installed to run MPEx.

Using MPEx: Membrane Protein Explorer: *Protein Profile*



0 saved buffers

Partitioning: water to bilayer

Select Wimley-White Scale: ● Octanol (Oct)

Plot Visibility: ☐ HF Moment

Select Mode: ● Locate

Window Size: 19

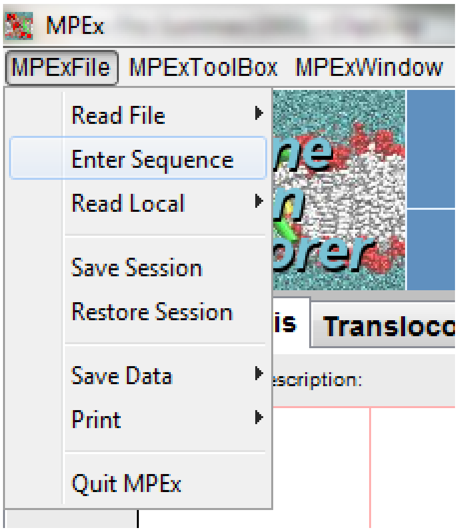
3) Launch the MPEx Program

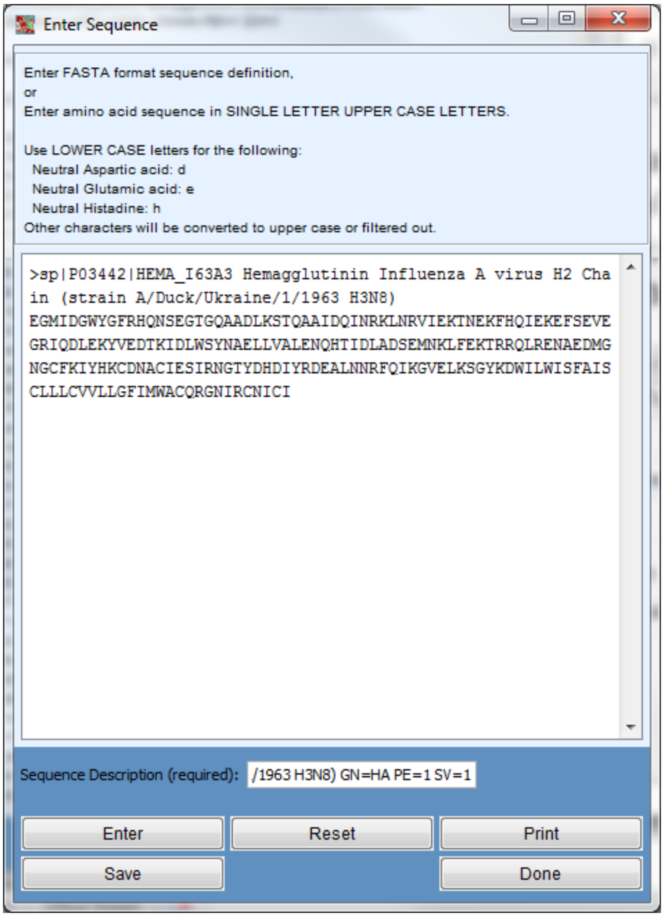
Using MPEx: Membrane Protein Explorer: *Protein Profile*

4) To obtain the whole protein sliding window Wimley-White Interfacial Hydrophobicity Scale (WWIHS) profile

a) Enter the protein sequence: Click MPExFile> Enter Sequence

b) Paste the FASTA sequence into the Window. Use CNTL-V in windows as right-click is not active.





Enter FASTA format sequence definition,
or
Enter amino acid sequence in SINGLE LETTER UPPER CASE LETTERS.

Use LOWER CASE letters for the following:
Neutral Aspartic acid: d
Neutral Glutamic acid: e
Neutral Histadine: h
Other characters will be converted to upper case or filtered out.

```
>sp|P03442|HEMA_I63A3 Hemagglutinin Influenza A virus H2 Cha
in (strain A/Duck/Ukraine/1/1963 H3N8)
EGMIDGWYGFRHQNSEGTGQAADLKSTQAAIDQINRKLNRVIEKTNEKFHQIEKEFSEVE
GRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLADSEMNKLFEKTRRQLRENAEDMG
NGCFKIYHKCDNACIESIRNGTYDHDIYRDEALNNRFQIKGVELKSGYKDWILWISFAIS
CLLLCVVLLGFIMWACQRGNIRCNICI
```

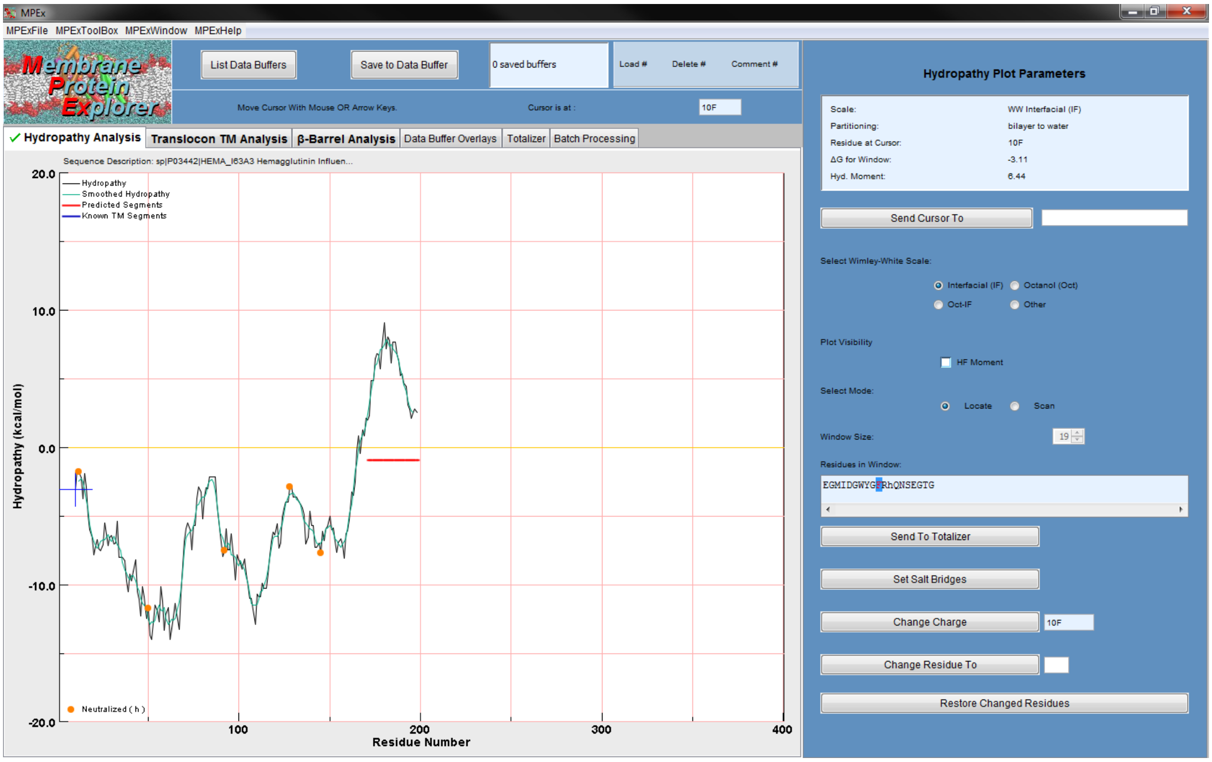
Sequence Description (required): /1963 H3N8) GN=HA PE=1 SV=1

c) Click “Enter” or “Done”

Using MPEx: Membrane Protein Explorer: *Protein Profile*

d) Click hydropathy analysis tab to see the results

e) At the right click “Interfacial (IF)” button for WWIHS



0 saved buffers

Cursor is at : 10F

Sequence Description: sp|P03442|HEMA_I63A3 Hemagglutinin Influen...

Scale: WW Interfacial (IF)

Partitioning: bilayer to water

Residue at Cursor: 10F

ΔG for Window: -3.11

Hyd. Moment: 6.44

Select Wimley-White Scale: ● Interfacial (IF) ○ Octanol (Oct) ○ Oct-IF ○ Other

Plot Visibility ☐ HF Moment

Select Mode: ● Locate ○ Scan

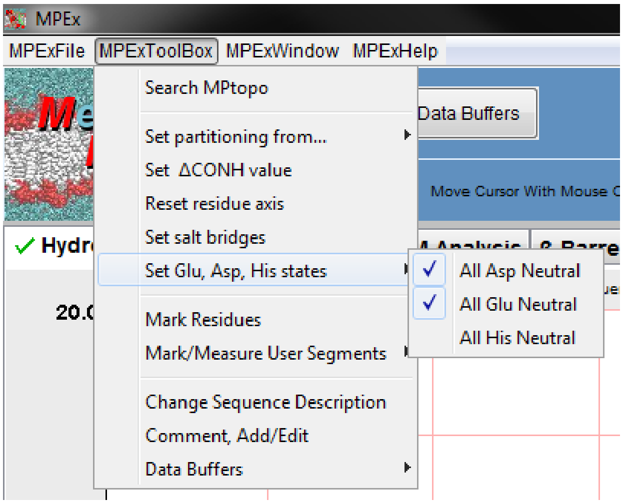
Window Size: 19

Residues in Window: EGMIDGWYGFRhQNSEGTG

Change Charge 10F

e) For low pH results, click MPExToolBox> Set Glu,Asp,His states

f) Set Glu and Asp to neutral (checked). Set His to charged (unchecked)

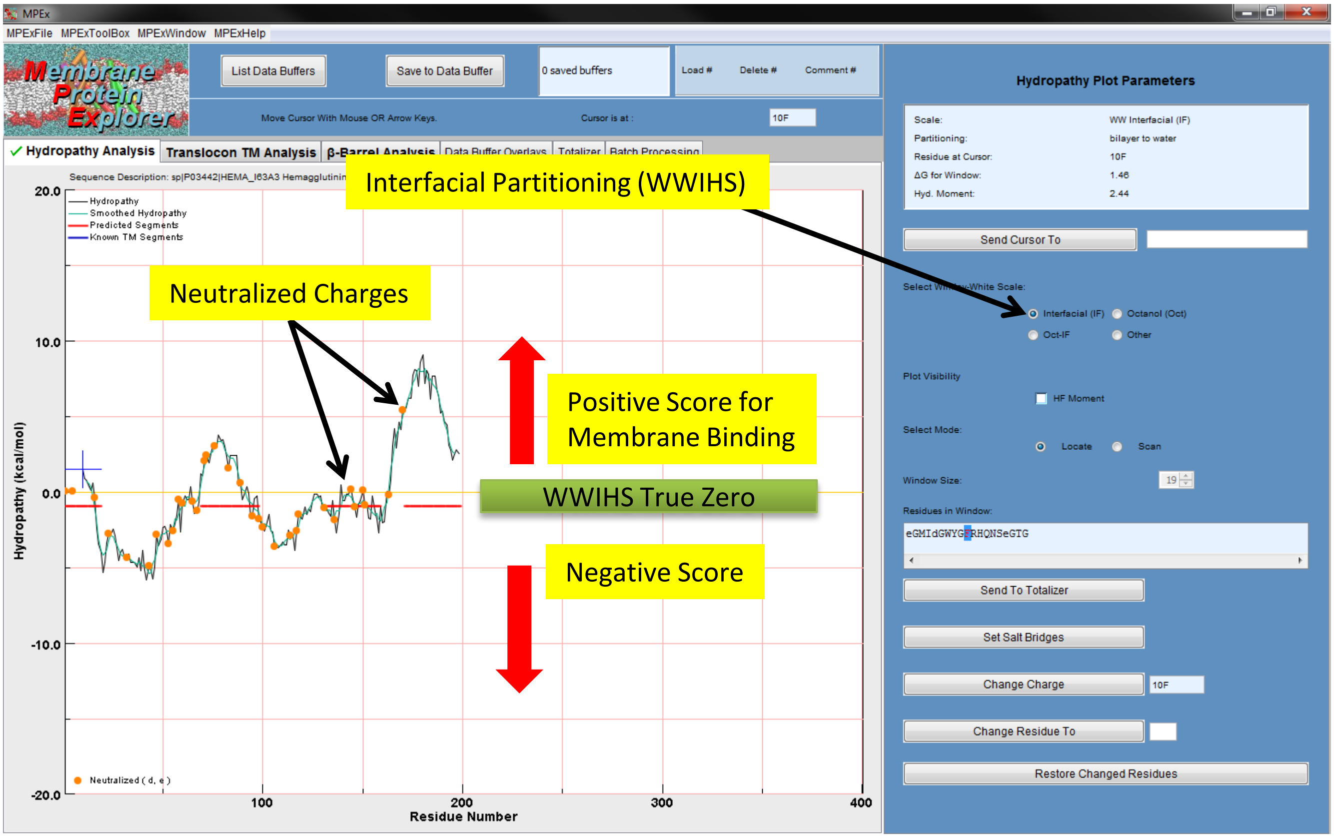


✓ All Asp Neutral

✓ All Glu Neutral

All His Neutral

Using MPEx: Membrane Protein Explorer: *Protein Profile*



Interfacial Partitioning (WWIHS)

Neutralized Charges

Positive Score for Membrane Binding

WWIHS True Zero

Negative Score

Sequence Description: sp|P03442|HEMA_I63A3 Hemagglutinin

0 saved buffers

Move Cursor With Mouse OR Arrow Keys.

Cursor is at : 10F

Hydropathy Plot Parameters

Scale: WW Interfacial (IF)
Partitioning: bilayer to water
Residue at Cursor: 10F
ΔG for Window: 1.46
Hyd. Moment: 2.44

Select Wimley-White Scale: ● Interfacial (IF) ○ Octanol (Oct) ○ Oct-IF ○ Other

Plot Visibility: ☐ HF Moment

Select Mode: ● Locate ○ Scan

Window Size: 19

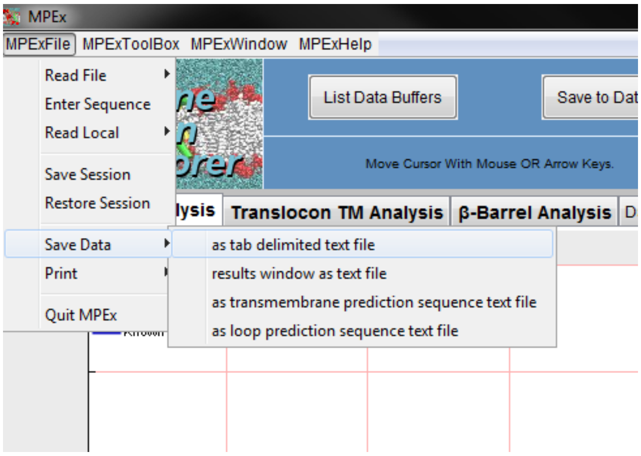
Residues in Window: eGMIdGWYGFRHQNSeGTG

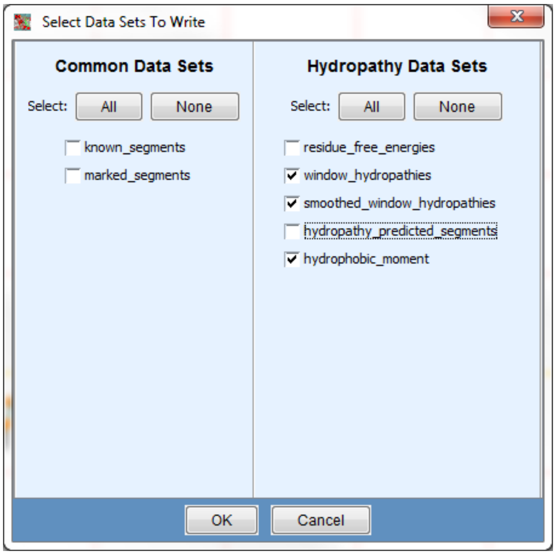
Change Charge: 10F

f) Segments above zero indicate a propensity to interact with membrane interfaces

g) Neutralized Glu and Asp are indicated by dots

Using MPEx: Membrane Protein Explorer: *Protein Profile*





Common Data Sets

☐ known_segments
☐ marked_segments

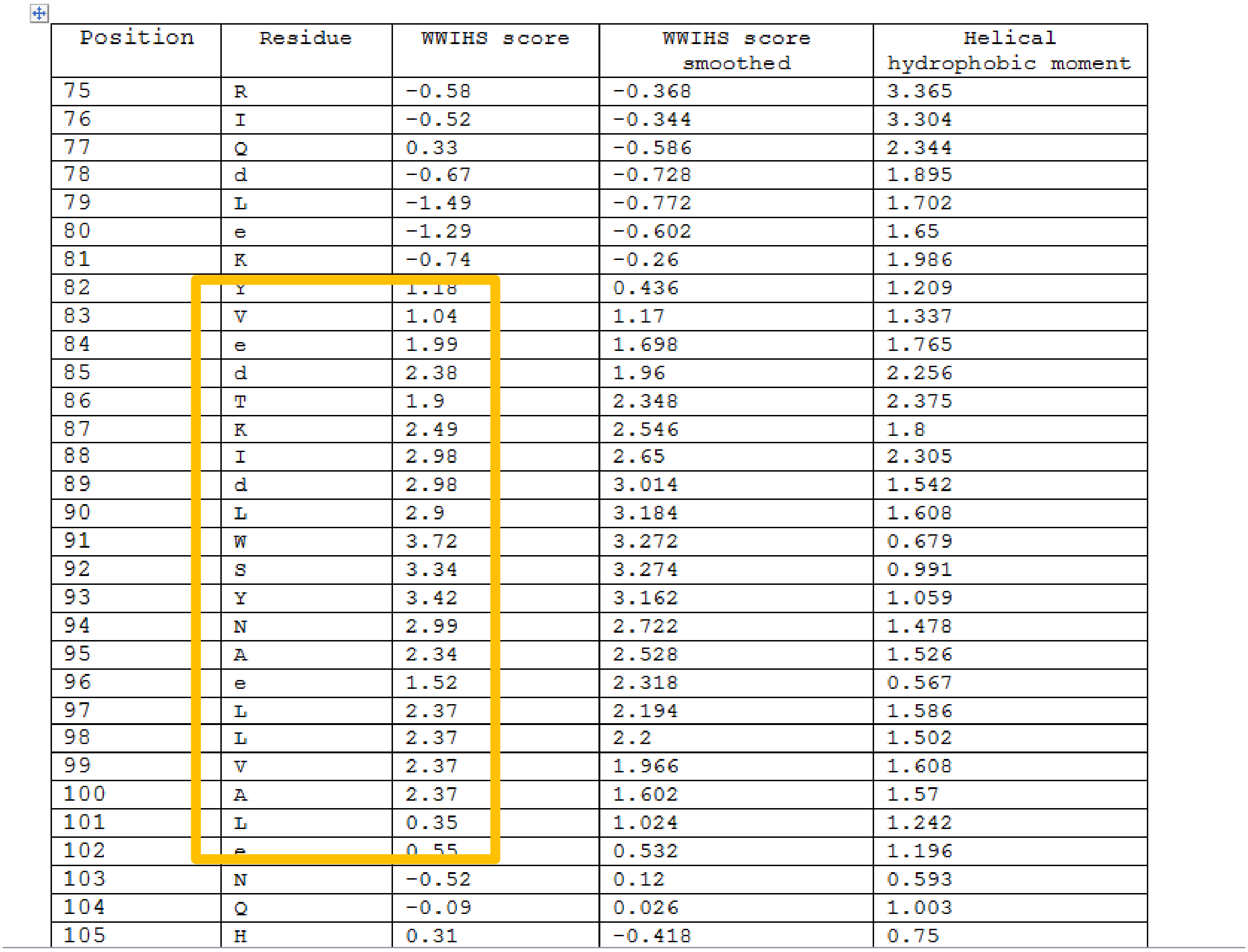
Hydropathy Data Sets

☐ residue_free_energies
☑ window_hydropathies
☑ smoothed_window_hydropathies
☐ hydropathy_predicted_segments
☑ hydrophobic_moment

h) To save profile as a text file for analysis and plotting:
Click MPExFile>Save Data> as tab delimited text file.

i) Select "Window_hydropathies". Optional data sets:
"Smoothed_Window_hydropathies" and "Hydrophobic_moment"

Using MPEx: Membrane Protein Explorer: *Protein Profile*

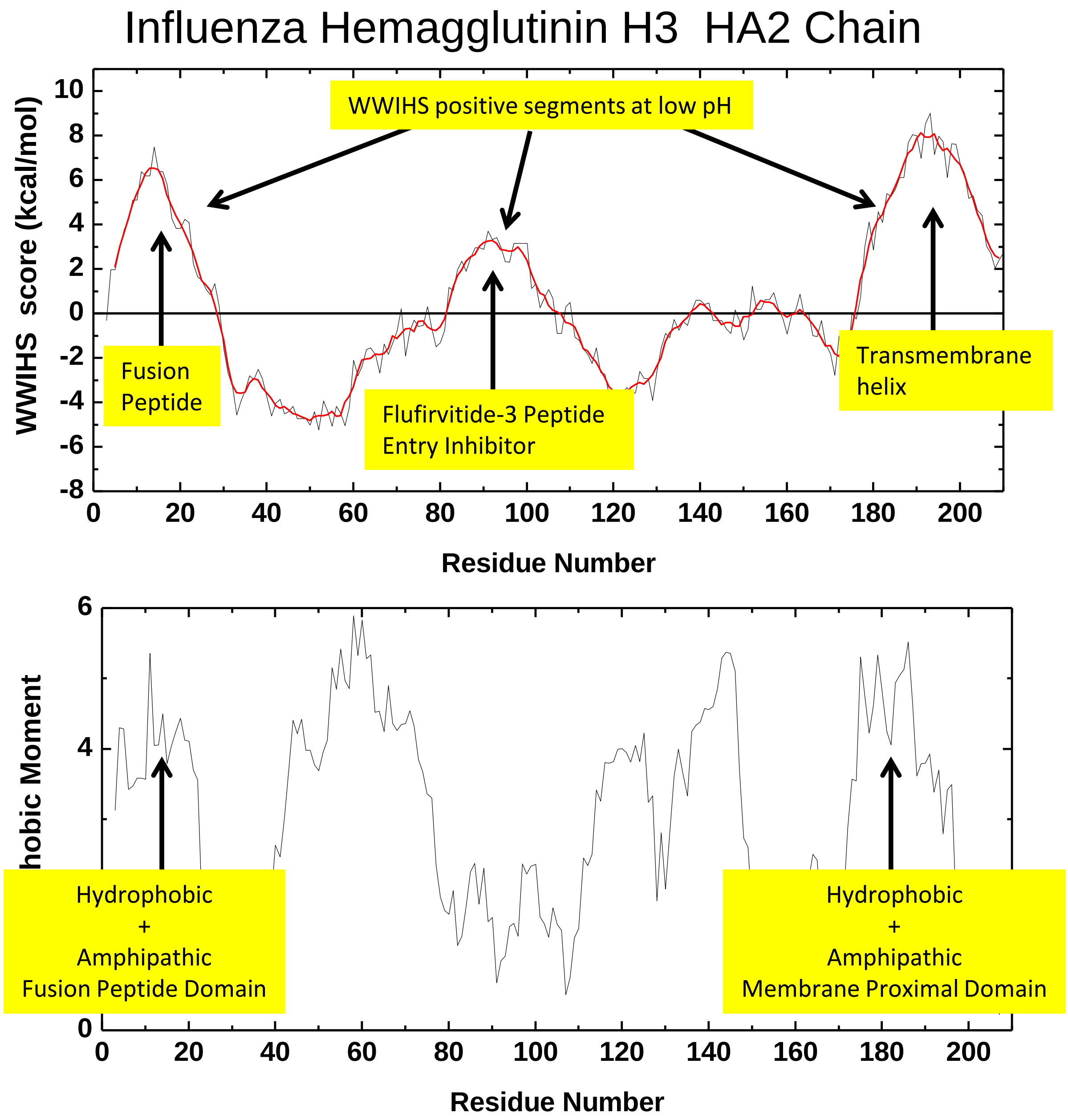


Position	Residue	WWIHS score	WWIHS score smoothed	Helical hydrophobic moment
75	R	-0.58	-0.368	3.365
76	I	-0.52	-0.344	3.304
77	Q	0.33	-0.586	2.344
78	d	-0.67	-0.728	1.895
79	L	-1.49	-0.772	1.702
80	e	-1.29	-0.602	1.65
81	K	-0.74	-0.26	1.986
82	Y	1.18	0.436	1.209
83	V	1.04	1.17	1.337
84	e	1.99	1.698	1.765
85	d	2.38	1.96	2.256
86	T	1.9	2.348	2.375
87	K	2.49	2.546	1.8
88	I	2.98	2.65	2.305
89	d	2.98	3.014	1.542
90	L	2.9	3.184	1.608
91	W	3.72	3.272	0.679
92	S	3.34	3.274	0.991
93	Y	3.42	3.162	1.059
94	N	2.99	2.722	1.478
95	A	2.34	2.528	1.526
96	e	1.52	2.318	0.567
97	L	2.37	2.194	1.586
98	L	2.37	2.2	1.502
99	V	2.37	1.966	1.608
100	A	2.37	1.602	1.57
101	L	0.35	1.024	1.242
102	e	0.55	0.532	1.196
103	N	-0.52	0.12	0.593
104	Q	-0.09	0.026	1.003
105	H	0.31	-0.418	0.75

j) These data can be used to identify WWIHS positive segments.

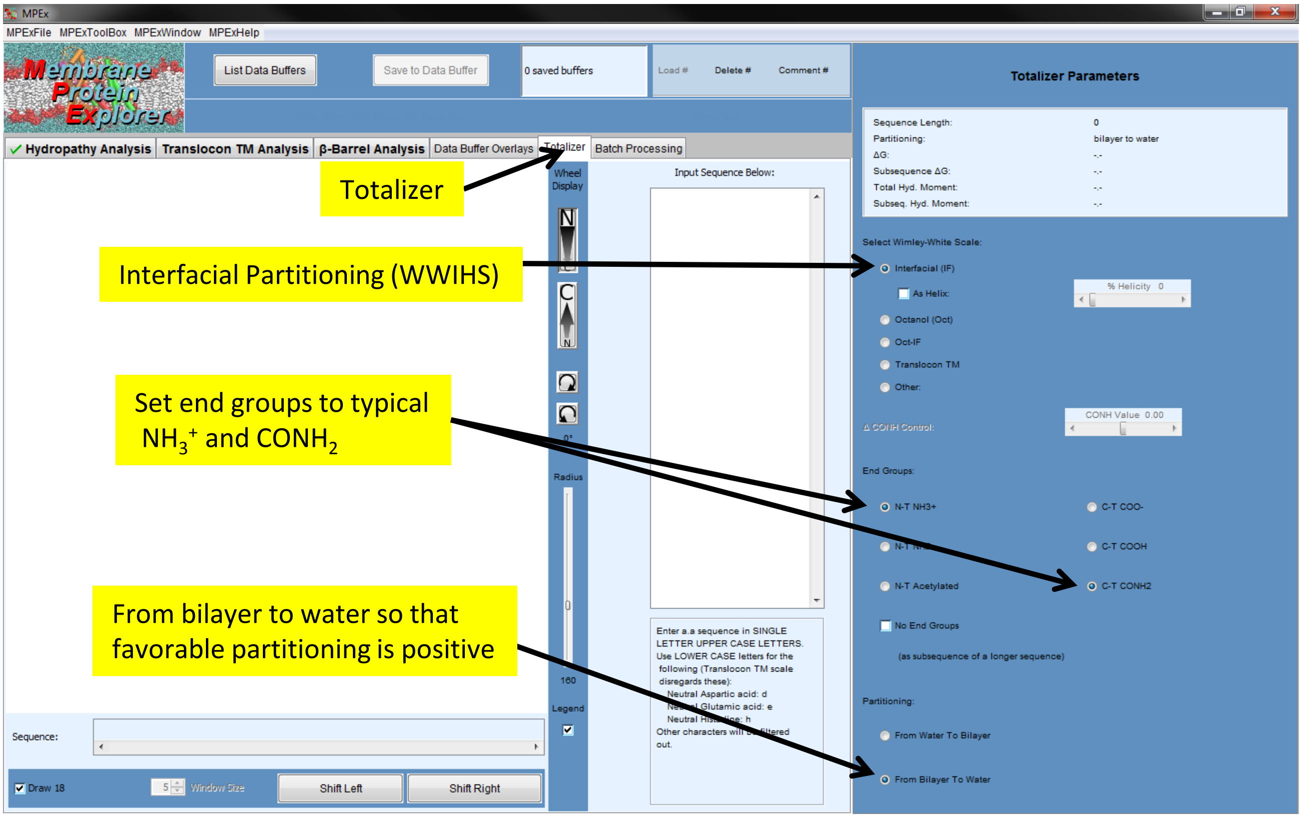
k) lower case E,D,H,K,R indicates that the residue is uncharged.

Using MPEx: Membrane Protein Explorer: *Protein Profile*



Using MPEx: Membrane Protein Explorer: *Peptide Analysis*

Analysis of individual peptides is done in Totalizer mode



Totalizer

Interfacial Partitioning (WWIHS)

Set end groups to typical NH_3^+ and $CONH_2$

From bilayer to water so that favorable partitioning is positive

0 saved buffers

Sequence:

☑ Draw 18

5 Window Size

☑ Legend

Enter a.a sequence in SINGLE LETTER UPPER CASE LETTERS. Use LOWER CASE letters for the following (Translocon TM scale disregards these):
Neutral Aspartic acid: d
Neutral Glutamic acid: e
Neutral Histidine: h
Other characters will be filtered out.

Totalizer Parameters

Sequence Length:	0
Partitioning:	bilayer to water
ΔG:	-.-
Subsequence ΔG:	-.-
Total Hyd. Moment:	-.-
Subseq. Hyd. Moment:	-.-

Select Wimley-White Scale:
● Interfacial (IF)
☐ As Helix:
% Helicity 0
○ Octanol (Oct)
○ Oct-IF
○ Translocon TM
○ Other:

Δ CONH Control:
CONH Value 0.00

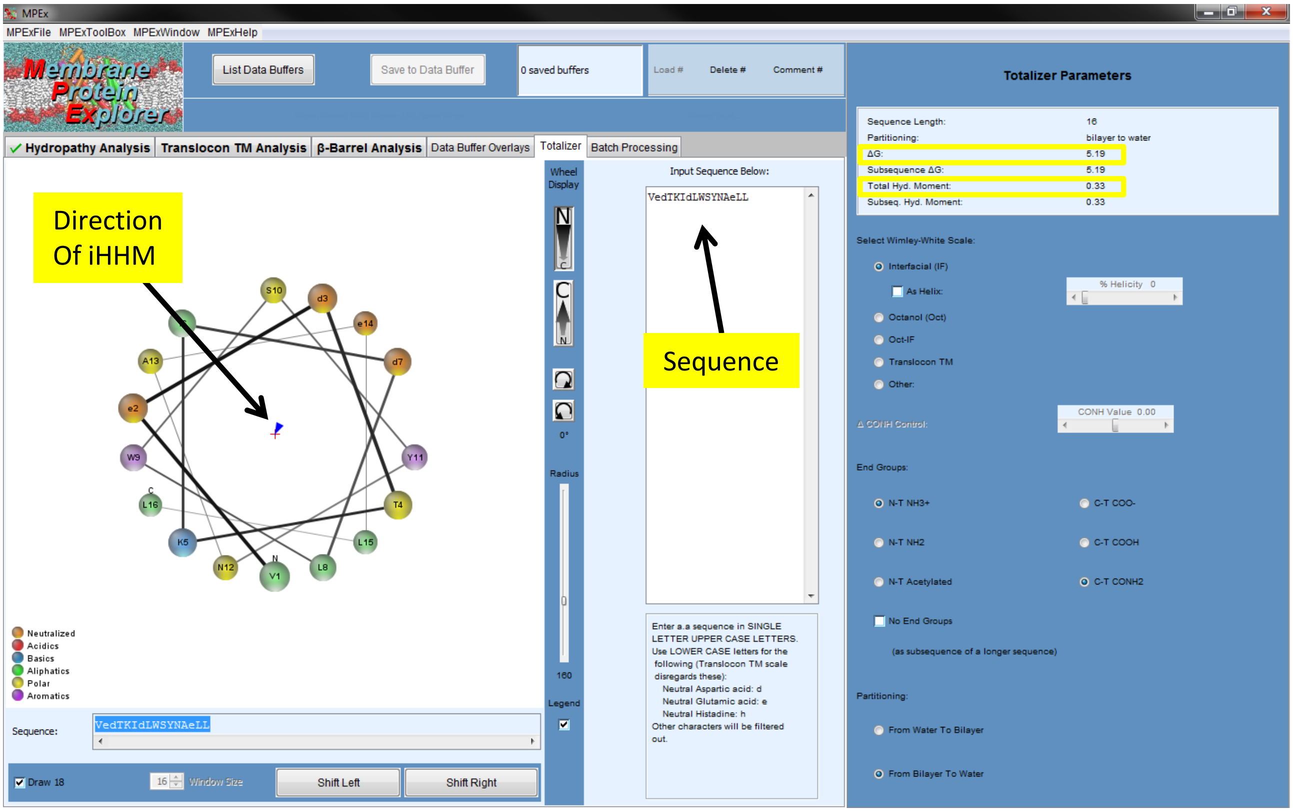
End Groups:
● N-T NH3+
○ C-T COO-
○ N-T NH2
○ C-T COOH
○ N-T Acetylated
● C-T CONH2
☐ No End Groups
(as subsequence of a longer sequence)

Partitioning:
○ From Water To Bilayer
● From Bilayer To Water

5) To obtain the Wimley-White Interfacial Hydrophobicity Scale score for a peptide sequence

a) Launch MPEx, Click “totalizer” tab.
b) Click “Interfacial (IF) for WWIHS”
c) Set end groups. Typical: NH_3^+ and $CONH_2$
d) Set partitioning from bilayer to water (interacting sequences will have positive scores).

Using MPEx: Membrane Protein Explorer: *Peptide Analysis*



Direction Of iHHM

Sequence

0 saved buffers

Input Sequence Below: VedTKIdLWSYNAeLL

Enter a.a sequence in SINGLE LETTER UPPER CASE LETTERS. Use LOWER CASE letters for the following (Translocon TM scale disregards these):
Neutral Aspartic acid: d
Neutral Glutamic acid: e
Neutral Histadine: h
Other characters will be filtered out.

Sequence: VedTKIdLWSYNAeLL

☑ Draw 18 16 Window Size

Legend ☑

Totalizer Parameters

Sequence Length:	16
Partitioning:	bilayer to water
ΔG:	5.19
Subsequence ΔG:	5.19
Total Hyd. Moment:	0.33
Subseq. Hyd. Moment:	0.33

Select Wimley-White Scale:
● Interfacial (IF)
☐ As Helix: % Helicity 0
○ Octanol (Oct)
○ Oct-IF
○ Translocon TM
○ Other:

Δ CONH Control: CONH Value 0.00

End Groups:
● N-T NH3+ ○ C-T COO-
○ N-T NH2 ○ C-T COOH
○ N-T Acetylated ● C-T CONH2
☐ No End Groups
(as subsequence of a longer sequence)

Partitioning:
○ From Water To Bilayer
● From Bilayer To Water

e) Enter sequence, or insert with CNTL-V. Capital D,E,H,K,R are charged. Lower case letters signify uncharged residues

f) For low pH peptide membrane interaction use lower case ***d*** and ***e*** and upper case H,K and R

Totalizer Results:
The WWIHS score is “ΔG”. Positive is favorable.
The interfacial Helical Hydrophobic Moment (iHHM) is “Total Hydrophobic Moment”. iHHM is always positive, but larger is more favorable.