Characterization of the M2 Proton Channel Functional Mechanism from Influenza A Obtained from Lipid Bilayer Preparations

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Brief Outline

- Intro to M2 and Influenza
- Intro to Oriented Sample solid state NMR
- M2 structure Determination in Lipid Bilayers
- Structural Comparisons
- Magic Angle Spinning solid state NMR Results
- The role of HxxxW in Proton Conductance

The “Spanish Flu” Pandemic of 1918-1919

- 1/3 of the world’s population was infected
- 3% of the world’s population died (50 to 100 million people)
- In the US, 28% of the population was infected
- In the US, 500,000 to 650,000 died
- Most fatalities were healthy young adults
Influenza A Viral Life Cycle

Adapted from Lamb & Krug, 1996
Influenza A Viral Life Cycle

1. Attachment of viral surface protein (HA) to host cell receptor
2. Fusion of viral and host cell membranes
3. Uncoating and release of viral RNA into the host cell cytoplasm

Rossman & Lamb, 2011
M2 Proton Channel
Amino Acid Sequence

Amino Terminus: 2 Cys residues form crosslinks

Aqueous Viral Exterior

Transmembrane Helix essential for proton conductance

Viral Membrane

Amphipathic Helix that binds lipid membrane surface

Aqueous Viral Interior

Carboxy-Terminus: M1 Binding Domain

M2 Proton Channel
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Aqueous Viral Interior

Carboxy-Terminus: M1 Binding Domain
M2 Conductance Domain

- Liposomal Assays show H⁺ conductance similar to full length protein & similar sensitivity to amantadine.

- Similar to Schnell & Chou, 2008 The conductance domain is shown to be a tetramer running at a slightly higher molecular weight that the predicted 19 kDa.

Emily Peterson and David Busath, BYU
Some of the Data for the M2 Conductance Domain Proton Channel Structure

• A tetrameric structure that shows some dimer of dimer character, but only subtle evidence in the OS ssNMR spectra.
M2 Proton Channel Characterized by ssNMR in DOPC/DOPG Lipids

The structure restrained primarily by orientational restraints and refined using restrained MD all in the same lipid bilayer.

The structure of the H37 tetrad and Trp41 tetrad were refined using QM/MM calculations.

Sharma et al., 2010 Science
Waters & Lipids Associated with the M2 Conductance Domain Structure

- Viral Interior
- Viral Exterior

- Scarce Water in pore due to Trp41 residues in the pore
- Abundant Water in pore due to Gly 34 residues in the pore
- His-His+ Pair

- Scarce Water in pore due to Trp41 residues in the pore – the primary gate for proton conductance
His37 Labeled Full–Length M2 (H57,90Y)

50 ms DARR spectra @ -10° C
In DOPC/DOPE as in PDB: 2L0J

Miao et al., 2015 Structure

>Dimer of Dimer?

>>Additional Complexity
Even More Complexity

Figure 5.1 Aromatic regions of 2D $^{13}$C-$^{13}$C correlation spectra with 50 ms mixing time at 243K on His37 labeled M2FL as a function of pH. Short-distance correlations are labeled in green in (B); The additional neutral conformation t5 is labeled in (B).

$^{13}$C-$^{13}$C Correlation Spectra of His37 at lower pH

Miao et al., *Structure*, 2015
His37 pH Titration of Full Length M2 in DOPC/DOPE Bilayers.

pH4.5
pH5.8
pH6.2
pH6.6
pH7.3
pH8.8

\[ { }^{15}\text{N (ppm)} \]

\[ \text{Charge} \]

\( \text{pK}_a \text{s: } 6.3 \pm 0.1 \)
\( 6.3 \pm 0.1 \)
\( 5.5 \pm 0.3 \)

Broadened Lines \( \Rightarrow \) Dynamics & Heterogeneity
>>Cooperative Protonation of His\textsubscript{37}

Miao et al., *Structure*, 2015
$^{15}\text{N}$ Spin Echo Spectra of pH 6.2 spectra

Miao et al., *Structure*, 2015
Both $\pi$ & $\tau$ uncharged states observed at high pH

$^{13}\text{C}$ (ppm)

NC zf TEDOR Spectra
1 ms mixing time
pH 7.3 & -10° C
Full Length M2

Miao et al., Structure, 2015
NC Spectra of the His37 at pH 7.3 & 6.2

Stable Structural Heterogeneity

Miao et al., *Structure*, 2015
Figure 5.1 Aromatic regions of 2D $^{13}$C-$^{13}$C correlation spectra with 50ms mixing time at 243K on His37 labeled M2FL as a function of pH. The four aromatic regions of 2D $^{13}$C-$^{13}$C correlation spectra with 50ms mixing time at 243K on His37 labeled M2FL as a function of pH. The four aromatic regions are labeled in (A) pH 4.5, (B) pH 5.8, (C) pH 6.2, (D) pH 6.6, (E) pH 7.3, and (F) pH 8.8.

- **Imidazole-Imidazolium H-bonded pairs**
- **(τ tautomer)**
- **(π tautomer)**
- **(charged state)**
His Tetrad Chemistry Driven by Charge Delocalization, Structural Stability

Solvated His-His+

Separated Charges: No H-Bond

Dong et al., Chem. Sci. RSC, 2013
His-His$^+$ Short H-bonds Confirmed in Full Length M2

His$^+$ protons exchange with water

$^1$H (ppm)

10

15

193 ppm

188 ppm

His-His$^+$ states exchange with H$_2$O-His$^+$ states

Multiple His-His$^+$ States

His-His$^+$ H-bonds associated with high $^1$H and $^{15}$N frequencies

Miao et al., *Structure*, 2015

HN HETCOR
500 µs contact time
pH 6.2
-10° C

$t$
Some Limits on the Exchange Rates

Hydronium attack on the neutral His

In $^1$H dimension:
4800 Hz > Exchange Rate > 0.2 Hz

In $^{15}$N dimension
300 Hz > Exchange Rate
Evidence for Short H-Bonds near Physiological Temperature

At 23°C, 15N frequencies associated with short H-bonds are present.

At 23°C, the His-His+ crosspeaks are not directly observed, but must be present since resonances at 190 ppm are observed.

The stability of the multiple states suggests:
- these states are not dictated by sidechains
- not by the very stable backbone structure
- but by the oligomeric helix packing

Miao et al., *Structure*, 2015
Evidence for both Exchange and Distances in the His37 Tetrad

Here exchange and distances from just one of the charged state (Ch1) are identified.
Sidechain Structural Dynamics at the TM – TM Interface
An Explanation for His-His+ Heterogeneity
HN Spectra of the His37 at pH 6.2 at -10°C (red) & 23°C (blue) – M2

Miao et al., 2015 Structure
Waters & Lipids Associated with the M2 Conductance Domain Structure

- **Viral Interior**
- **Viral Exterior**

**Abundant Water in pore due to Gly 34 residues In the pore**

**His-His+ Pair**

**Scarce Water in pore due to Trp41 residues In the pore – the primary gate for proton conductance**
Evidence for a Futile Cycle

A Futile Cycle in which hydronium attacks from the external pore and the proton is reabsorbed by a water in the aqueous pore.

Attack by hydronium appears to be sub-msec timescale.

Conductance is known to be \( \sim 100/s \).

Maybe even less frequent at \(-10^\circ C\).
M2 Conductance Mechanism

- Hydronium ion attack of the +2 state resulting in a +3 state and breaking a short H-bond.

- A proton can be absorbed by water from either the external or internal pore.

- If the proton is absorbed into the external pore a Futile Cycle results.

- If the proton is absorbed into internal pore a Conductance Cycle results.

Miao et al., *Structure*, 2015
Cholesterol Stabilizes the Amphipathic Helix

Mixed sample of: 13C Leucine FLM2 and 13C Phenylalanine FLM2

Slices through Leu Ca

Distances in Red represent distances From the 2L0J M2 structure

Ekanayake et al., in press, BJ
**Cholesterol and M2FL**

A Typical Cholesterol binding pocket with:
- C3 close to Phe 54
- Cholesterol in Helix-Helix Crevice
- Cholesterol close to palmitylation site, Cys50.

Ekanayake et al., in press, BJ
Fourth & Final Set of Conclusions:

- M2 has a unique Histidine Tetrad that is a His–His$^+$ Dimer of Dimers Structure
- M2 Full Length Protein Displays Exchange and Dynamic Processes to Facilitate Proton Exchange
- M2 Conductance is a Combination of a Futile and Conductance Cycles
- Biological Chemistry Requires a Molecular Framework on which to Hang the Chemistry & Dynamics to Facilitate the Chemistry
- Solid State NMR can Uniquely Characterize the Structural and Dynamic Details in Membrane Proteins in a variety of model environments and in cell membranes
Just Some of those who contributed so much to my lab