Helical Membrane Protein Biophysics: Oriented Sample Solid State NMR

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&
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Single Site resonance in fd Filamentous Viral Coat Protein

Figure 1. $^{15}$N NMR spectra of $[^{15}\text{N}]_6$Trp-26-labeled fd in solution. These spectra were obtained at 15.2 MHz on a home-built double resonance spectrometer: (A) chemical shift powder pattern for an unoriented sample (150 mg/mL, pH 6; $\sigma_{33} = 147$ ppm, $\sigma_{22} = 108$ ppm, $\sigma_{11} = 39$ ppm); (b) chemical shift spectrum of an oriented sample (45 mg/mL, pH 8). The contour plot represents the data from a two-dimensional separated local field experiment using off-resonance $^1$H irradiation to suppress $^1$H-$^1$H interactions; the projection of the dipolar splitting is aligned on the left side with $|\Delta \nu_d| = 1.0$ kHz when corrected for the experimental scaling factor. The spin-interaction tensors are drawn schematically in the molecular frame of a tryptophan side chain. $\sigma_{ll}$ are the principal elements of the chemical shift tensor and $\nu_{ij}$ is the component of the dipolar interaction parallel to the N-H bond.


Protein Structure by Solid-State NMR

T. A. Cross and S. J. Opella*

Department of Chemistry, University of Pennsylvania
Philadelphia, Pennsylvania 19104
Received September 23, 1982
Helices

- Residues & Atoms per turn:
  - $\alpha$-Helix: 3.6 residues & 13 atoms ($3.6_{13}$ helix)
  - $\beta$-Helix: 3.2 residues & 10 atoms ($3.2_{10}$ helix)
  - $\gamma$-Helix: 4.4 residues & 16 atoms ($4.4_{16}$ helix)

$3_{10}$ helix (i to $i+3$ hydrogen bonds)

\[
\text{H-C-N-CHR-C-N-CHR-C-N-CHR-C-N-CHR-C-N-H}
\]

\[
\begin{array}{ccccccc}
\text{O} & \text{H} & \text{O} & \text{H} & \text{O} & \text{H} & \text{O} \\
1 & 2 & 3 & 4 & 5 & \\
\end{array}
\]

$\alpha$ helix (i to $i+4$ hydrogen bonds)

\[
\text{H-C-N-CHR-C-N-CHR-C-N-CHR-C-N-CHR-C-N-H}
\]

\[
\begin{array}{ccccccc}
\text{O} & \text{H} & \text{O} & \text{H} & \text{O} & \text{H} & \text{O} \\
1 & 2 & 3 & 4 & 5 & \\
\end{array}
\]

$\pi$ helix (i to $i+5$ hydrogen bonds)

\[
\text{H-C-N-CHR-C-N-CHR-C-N-CHR-C-N-CHR-C-N-H}
\]

\[
\begin{array}{ccccccc}
\text{O} & \text{H} & \text{O} & \text{H} & \text{O} & \text{H} & \text{O} \\
1 & 2 & 3 & 4 & 5 & \\
\end{array}
\]
Helices

3_10 Helix
\[ \phi = -68 \]
\[ \psi = -17 \]

\( d = \) peptide plane tilt angle wrt helix axis
\[ \delta = +18.9 \]

\( \delta = +11.8 \)

\( \delta = -2.2 \)

a Helix
\[ \phi = -65 \]
\[ \psi = -40 \]

p Helix
\[ \phi = -57 \]
\[ \psi = -70 \]

Kim & Cross (2004) JMR.
Helices & PISA Wheels Calculated for 30° Tilt to $B_0$

PISEMA Simulations

$^1\text{H} - ^{15}\text{N}$ Dipolar Coupling (kHz)

$^1\text{H} - ^{15}\text{N}$ Dipolar Coupling (kHz)

$^1\text{H} - ^{15}\text{N}$ Dipolar Coupling (kHz)

Helical Wheels

Kim & Cross, JMR 2004
Rhamachandran Diagram

Nonbonded contact radius

β-sheet

$3_{10}$ helix

left-handed α-helix
	right-handed α-helix
The Rhamachandran-delta diagram

- For uniform helical structures: Lines of constant peptide plane tilt angle wrt helix axis

Kim & Cross, JMR 2004
Other Helices Occur - $3_{10}$ Helices: Voltage Gated 2-pore channel – PDB 5E1J

Why use a $3_{10}$ helix?

Observed PISA Wheels: $\varphi = 9 \pm 4^\circ$; $\varphi = -60^\circ$, $\varphi = -45^\circ$

Page et al., Structure 2008
Membrane Protein Environment

<table>
<thead>
<tr>
<th>Membrane Environment</th>
<th>Aqueous Environment</th>
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<tbody>
<tr>
<td>Dielectric Constant:</td>
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<td>[H2O]:</td>
<td>~10^-6 to 55 M</td>
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<tr>
<td>Fluidity S_{cd}:</td>
<td>0 - 0.4</td>
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<tr>
<td>Lateral Pressure:</td>
<td>1 to 300 atm</td>
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<td></td>
<td>1 atm</td>
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Nobel Laureate Christian Anfinsen’s thermodynamic hypothesis states “…that the native conformation (of a protein) is determined by the totality of inter-atomic interactions and hence by the amino acid sequence in a given environment.”

Science July 20, 1973

Electric and Chemical potentials across the membrane

Variable Lipid and Protein Composition

Protein Structure and Function are modulated by the Membrane Environment & Lipid Composition

Cross (2018) Structure 26:2-4
Helical Torsion Angles

- The difference between $\psi$ angles of $-40^\circ$, $-65^\circ$ and $-45^\circ$, $-60^\circ$

\[ \alpha\text{-helix}_{\text{H}_2\text{O}} \quad \alpha\text{-helix}_{\text{membrane}} \]
Torsion Angle Data for Membrane Proteins from x-ray crystallographic structures

- Each ellipse contains 90% of the TM helical torsion angles

- Since the crystal lattice is not a membrane environment we expect less torsion angle variation in structural characterizations performed on membrane proteins in lipid environments

Bacteriorhodopsin Structures:
- 1C3W: 1.55Å
- 1QHJ: 1.9Å
- 1AP9: 2.35Å
- 1BM1: 3.5Å

Page et al., 2008 Structure
Dependence of PISA Wheels on Local Conformation

\[ \begin{align*}
\phi &= -60 \pm 0^\circ \\
\psi &= -45 \pm 0^\circ
\end{align*} \]

\[ \begin{align*}
\phi &= -60 \pm 4^\circ \\
\psi &= -45 \pm 4^\circ
\end{align*} \]

\[ \begin{align*}
\phi &= -60 \pm 8^\circ \\
\psi &= -45 \pm 8^\circ
\end{align*} \]

Page et al., 2007 MRC
In 2007 OS ssNMR Spectra of $^{15}$N Trp & Met Labeled Diacyl Glycerol Kinase

Li et al., JACS 2007
In 2009 – A Solution NMR Structure in Detergent Micelles

Very Poor Agreement with the PISEMA Data Obtained in Lipid Bilayers.

Van Horn et al., (2009)

Murray et al., Biophys. J. 2014
In 2013 X-Ray structures in Lipidic Cubic Phase: WT DgkA – A& C

3 Met Labeled DAGK

5 Trp Labeled DAGK

Structures A and C in the trimer are in near perfect agreement with the PISEMA Data

Li et al (2013) Nature

Murray et al., Biophys. J. 2014
In 2013 X-Ray structures in Lipidic Cubic Phase: Structures A&B

- Structure B displays significant deviations from the lipid bilayer results.
- Structure B has multiple thermal stabilizing mutations - one of these was A41C in the TM domain.

Li et al (2013) Nature
First Set of Conclusions

- The scatter in the observed data for TM helices is restricted to ± 4° in $\chi$ (peptide plane tilt)
  ± 8° in $\phi/\psi$ torsion angles
  Modest chemical shift tensor element magnitude and orientation variation except for Glycine.

- These OS ssNMR studies were the first studies to show the uniformity of helical structures in lipid bilayer environments – a feature that is now well (?) accepted in the crystallographic community.

- Distinction between of $3_{10}$ and $\alpha$-helices is clear

- This high resolution structural detail is available for validating structures obtained by other methods and in other environments.

- Solid State NMR can Uniquely Characterize the Structural and Dynamic Details in Membrane Proteins in a variety of model environments and in cell membranes
Mycobacterial Cell Wall

- Mycolic Acids
- Arabinan
- Galactan
- Peptidoglycan
- Cell Membrane
The Divisome of *Mycobacterium tuberculosis*

*Mtb* elongates from the poles unlike most bacteria and therefore requires coordination between an elongation complex and the divisome.

Only FtsZ, the protein that forms the Z-ring in dividing cells, binds to as many proteins as CrgA known to be a primary recruiter of proteins to the divisome and is a negative regulator of cell division.

Some *Mtb* Proteins Associated with Cell Division

- **Wag31** 260 aa
  - 46-48
- **FtsI** (PBPB, PBP3) 679 aa 1TM IDD
  - Septation xpeptidase
- **ChiZ** 165 aa 1 TM motifs
  - PG hydrolase IDD
- **CwsA** 145 aa
  - 1 TM motif IDD
- **CrgA** 93 aa
  - 2 TM motifs
- **PBPA** 491 aa
  - 1 TM
  - PG Synthesis
- **FtsZ** 379 aa
  - Implies IDD
  - Implies Partial Known Structure
- **FtsQ** 314 aa
  - 1 TM motif IDD
Many *Mtb* Divisome Proteins are Predicted to have IDDs

<table>
<thead>
<tr>
<th>ChiZ</th>
<th>Cytoplasmic</th>
<th>TM helices</th>
<th>Periplasmic</th>
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<td>TPALAVGQTL IAPVG</td>
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<table>
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<table>
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<tr>
<td>GFDQFNRRGKN</td>
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CrgA: A regulatory protein of *M. tuberculosis* cell division
Full Length CrgA from *Mtb* – SAMPI4 Spectra

MPKSKVRKKNDFTVSAVSRTPMKVKVGPSSVWFVSLFIGLMLIGLIWLVMFQLAAIGSQAPTALN
WMAQLGPWNYAIAFAMITGLLLTMRWH

Das et al., 2015 PNAS
Amino Acid Labeled CrgA

MPKSKVRKKN$_{10}$ DFTVSAVSRT$_{20}$ PMKVKGPS$_{30}$ VWFVSLFGL$_{40}$ MLIGLIWLMV$_{50}$ FQLAAGSQA$_{60}$
PTALNWMAQL$_{70}$ GPWNYAI$A_{80}$ FMITGLLLTM$_{90}$ RWHELH$H_{100}$

Temp: 13 °C
POPC POPG (liposome)
LPR: 100:1 (Molar)

Das et al., PNAS 2015
Dipolar Waves for the Two TM helices in CrgA

(a) CrgA TM1 Residue 31-52

(b) CrgA TM2 Residue 73-91

Das et al., PNAS 2015
CrgA from *Mtb* – Distance Restraints

$^{13}$C-$^{13}$C Correlation Spectra (50 & 25 ms)

Structure of the 2 TM helices

Das et al., 2015 PNAS
CrgA from \textit{Mtb} – $^{13}\text{C}-^{13}\text{C}$ DARR Spectra

Reverse Labeling: excluding Phe, Trp, Ser, Thr, Ile

- Blue: 300 ms
- Green: 700 ms
- Red: 1000 ms

G39Ca-T84Cb
L42Ca-A78Cb
M49Ca-A78Cb
M49Ca-Y75Cb
M49Ca-Y75Ce1

Das et al., 2015 PNAS
CrgA TM 2° and 3° Structure

Das et al., PNAS, 2015
Absolute vs. Relative Restraints

- Absolute restraints for secondary structure and structural orientation relate the protein to LAB frame.

- Relative restraints are particularly useful for tertiary and quaternary structure and relate one site to another in the protein.

$^{15}$N-$^1$H Dipolar Orientational Restraints ±0.5 kHz
$^{15}$N Anisotropic Chemical Shift ±5 ppm

Backbone-Backbone Distance Restraints ±0.5Å

Das et al., 2014 Adv. Biol. SSNMR
How Many Backbone Restraints does $^{15}$N Labeling Yield?

<table>
<thead>
<tr>
<th>Obs.</th>
<th>Pred.</th>
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<tr>
<td>32: 105°</td>
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<td>33: 230°</td>
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<tr>
<td>34: 285°</td>
<td>290°</td>
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<td>35: 20°</td>
<td>30°</td>
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<td>36: 145°</td>
<td>130°</td>
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<td>37: 270°</td>
<td>230°</td>
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<td>38: 310°</td>
<td>330°</td>
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<td>39: 60°</td>
<td>70°</td>
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<td>40: 195°</td>
<td>170°</td>
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<td>41: 245°</td>
<td>270°</td>
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<td>42: 20°</td>
<td>10°</td>
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<td>43: 125°</td>
<td>110°</td>
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<td>44: 210°</td>
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<td>45: 340°</td>
<td>310°</td>
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<tr>
<td>46: 30°</td>
<td>50°</td>
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<tr>
<td>47: 190°</td>
<td>150°</td>
</tr>
<tr>
<td>48: 290°</td>
<td>250°</td>
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</table>

Rotation Angle: 0°

Tilt Angle:

C: Ala 76, 78, 80:

D: Tilt = 15°

E: Tilt = 15°
How Many Backbone Restraints does $^{15}$N Labeling Yield?

- Recognizing that there is a helical pattern generates opportunities for additional restraints.

- Instead of treating the restraints from each peptide plane as independent of the other planes, they are, in fact, coupled by the Ca carbon.

- In other words knowing that residue 38 has a rotation angle relative to residue of 31 of $\pm 10^\circ$ is an important structural restraint!

- High resolution structures of helices and other secondary structure are determined. Distances are vitally important for packing the helical structure and or b-strands into a tertiary or quaternary structure.
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<td>41-47: 455°</td>
<td>36-42: 595°</td>
<td>35-41: 585°</td>
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<td>32-34: 275°</td>
<td>31-36: 515°</td>
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<td>l-l+4</td>
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<td>37-41: 335°</td>
<td>39-45: 640°</td>
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<td>33-37: 385°</td>
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<td>34-40: 630°</td>
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120 additional restraints
Dipolar Couplings Outside of the TM Helices

N-terminus
Intrinsically Disordered Domain

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<th>Dipolar Coupling (kHz)</th>
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<td>56</td>
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<td>57-73</td>
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- ~ Isotropic value

Inter-helical Loop

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<td>72</td>
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<td>73</td>
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- ~ Isotropic value

- Anisotropic Chemical Shifts Present the Same Story

- TM Helix 1

- TM Helix 2
Anisotropic Chemical Shifts Outside of the TM Helices

N-terminus

Intrinsically Disordered Domain

b Strand or Amphipathic Helix

~ Isotropic value

TM Helix 1

Inter-helical Loop

Helix extension

b Strand or Amphipathic Helix

~ Isotropic value

TM Helix 2

Anisotropic Chemical Shifts Present the Same Story as the Dipolar Interactions
Wild Type CrgA appears to be a Dimer

1-29 Deletion G44V WT

>> The stability of the dimer can be enhanced: A78V and G44V

25 kDa 20 kDa 15 kDa 10 kDa

w/o N-terminus 11mg WT 12mg

>> Only when gels were heavily loaded was the dimer observed in gels.
Possible Interfacial Model for N-terminal CrgA Dimerization

RT$_{20}$ PMKVKGPS$_{30}$

Lipid Head Group Environment

CrgA Monomer 1
C $\leftrightarrow$ N

Fatty Acyl Chain Environment

CrgA Monomer 2
N $\rightarrow$ C
K$_{23}$ VKV
Second Conclusion Slide

- PISA wheels and Dipolar waves together define precise rotation and tilt angles for the helices.

- Anisotropic chemical shifts and Dipolar Interactions can be used to identify Intrinsically Disordered Domains and other Structured Domains.

- The CrgA data for two helices fit remarkably well to a single PISA wheel pattern implying that the helical structures have the same tilt angle.

- Correlation Restraints provide additional restraints between the peptide planes resulting in a high resolution structure of the helical backbone.

- The combination of orientational and distance restraints is needed for high resolution structural characterization of transmembrane helices and their orientation with respect to their environment.
This is the Centennial Year of the “Spanish Flu” Pandemic of 1918-1919: NMR Spectroscopy of the M2 Protein from Influenza A Virus

- 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 particles to the host cell membrane.
2. Fusion of the viral envelope with the host cell membrane.
3. Entry 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 – essential for viral budding

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 than 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 only slight dimer of dimer character in the OS ssNMR spectra.

Sharma et al., 2010 Science
M2 Proton Channel Characterized by ssNMR in DOPC/DOPG Lipids

Viral Exterior

Viral Interior

Viral External Pore

Viral Internal Pore

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
$^{13}$C His37 Labeled Full Length M2 Protein Spectra at 7.3

$^{13}$C – $^{13}$C Correlation spectra 50 ms mixing time

- 4 states for the 4 histidines – lineshapes suggest further possible complexity.
- A possible explanation for the 3 t and 1p state:
  - 2 tau states forming a t–t H-bonded dimer
  - plus a p–p or an additional t–t pair

NC zf TEDOR Spectra Full Length M2
1 ms mixing time

Miao et al., Structure, 2015
$^{13}\text{C}-^{13}\text{C}$ Correlation Spectra of His37 at lower pH

Even More Complexity

50 ms DARR spectra @ -10°C

Miao et al., Structure, 2015
$^{15}$N His$_{37}$ Full Length M2 in DOPC/DOPE Bilayers.

From our complete set of titration data:

- $^{13}$C Chem. Shifts
- $^{15}$N Chem. Shifts
- $^{15}$N-$^{13}$C Dipolar
- $^{15}$N-$^{1}$H Dipolar

$pK_a$s: 6.3 ± 0.1
6.3 ± 0.1
5.5 ± 0.3

Heterogeneous broadening

$T_2^* = 1/(\pi\Delta\nu^*) = 2.6 \text{ ms}$
$Dn^* \sim 2 \text{ ppm}$

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

Stable Structural Heterogeneity

(charged state)

Miao et al., Structure, 2015
M2FL NC Spectra of the His37 at pH 6.2

- Imidazole-Imidazolium H-bonded pairs

Miao et al., *Structure*, 2015
His-His$^+$ Short H-bonds Confirmed in Full Length M2

His$^+$ protons exchange with water

$^1$H (ppm)

HN HETCOR
pH 6.2, -10°C (liquid crystal lipid bilayers)

His-His$^+$ states exchange with $\text{H}_2\text{O}$-His$^+$ states

Multiple His-His$^+$ States

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

3600 Hz

$^1$5N (ppm)

In $^1$5N dimension
300 Hz > Exchange Rate

Miao et al., Structure, 2015
Evidence for Short H-Bonds near Physiological Temperature

At 23°C $^{15}$N frequencies associated with short H-bonds are present

At 23°C the His-His+ crosspeaks are not directly observed, but must be present since resonces 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
So Where does this Heterogeneity come from?

.... Biology is messy
.... in part:

- Tetramer stability is dependent on van der Waals interactions
- Helical interface includes large hydrophobic side chains
- In MD runs - various torsional states – varying N-N distances from 2.7-2.9 Å
The Evidence for Imidazole-Imidazole Dimers is Now Overwhelming

- Cooperative proton binding
- Characteristic $^1$H chemical shifts approaching 20 ppm
- Characteristic protonated $^{15}$N chemical shifts up to 193 ppm
- Characteristic non-protonated $^{15}$N chemical shifts down to 235 ppm
- Spectral Heterogeneity consistent with the tetrameric structure that supports the dimer of dimer imidazole-imidazolium structure

Imidazole-imidazolium Hydrogen Bonds Under Attack in the M2 Proton Channel

Miao et al., *Structure*, 2015
His Tetrad Chemistry Driven by Charge Delocalization, Structural Stability

Solvated His-His+

Separated Charges: No H-Bond

Dong et al., Chem. Sci. RSC, 2013
Evidence for both Exchange and Distances in the His37 Tetrad

Here exchange and distances from just one of the charged state (Ch1) are identified.

Miao et al., 2015 Structure
$^{13}\text{C} \text{ Correlation Spectra &}$

$^{13}\text{C} - ^{13}\text{C} \text{ & } ^{15}\text{N} - ^{15}\text{N} \text{ Exchange Spectra}$

- $^{15}\text{N}$-Exchange Spectra: t to + interconversion
- $^{13}\text{C}$ Exchange Spectra: t to + interconversion
- Circled: Primary spin exchange peaks within a His 37 residue

Qin et al., *JCPB*, 2015
Characterizing the chemical exchange rate between water and the bridging imidazole-imidazolium hydrogen bond

- Dipolar dephasing of the imidazole N-H followed by chemical exchange at the magic angle with water protons

Fu et al., 2016, JACS
Characterization of the chemical exchange Rate

- The observed rate of H⁺ exchange with water increases by a factor of ~2.3 between pH 6.2 and pH 5.8.
- Previously said that the exchange rate was at a rate that is less than 4800 Hz at pH 6.2.
- The hydronium ion concentration increases by a factor of 2.50 between pH 6.2 and 5.8.

Fu et al., JACS, 2016
Qin et al., JCPB, 2015
M2FL NC Spectra of the His37 at pH 6.2

- Different states
- Exchange at Different Rates

$t_{SL} = 100 \text{ ms}$
$t_{SL} = 2000 \text{ ms}$

Fu et al., JACS, 2016
Miao et al., Structure, 2015
Conductance Mechanism: Imidazole-Imidazolium Dimer Mechanism

Viral Exterier
H^+

Viral Interior

Histidine-Locked State

Conducting State

Activated State

Acid Activation

Sharma et al., 2010 Science
M2 Proton Channel – A Small Helical Membrane Protein – Structure of the Conductance Domain – Residues 22-62

Viral Exterior

Viral Interior 40 lipids in van der Waals contact with the M2 tetramer

Sharma et al., 2010 Science
Full Length M2 Spectra from Influenza A

Full Length M2 Protein $^{13}$C-$^{13}$C Correlation Difference Spectrum 50-25 ms.

2L0J – Inter-helical and inter-residue Distances consistent with the Conductance Domain Structure

Miao et al., J. Biomol. NMR 2013

Miao et al., Angw. Chemie, 2013
Conductance Mechanisms

1. Monomeric Histidine Mechanism
   
   Hu et al., JACS 2012
   134:3703-3713
   • Conductance State is +1

2. Imidazole-Imidazolium Dimer Mechanism
   
   Sharma et al., 2010, Science

- Activated State is +2
- Conductance State is +3
Waters & Lipids Associated with the M2 Conductance Domain Structure

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

Viral Exterior

Futile Cycle

Conductance

Viral Interior

Miao et al., Structure, 2015
Implications for the Conductance Mechanism

- c1 torsional motion dominates the motions resulting from protonation and deprotonation, but for the Conductance mechanism both c1 and c2 torsional motions are required.

2a. Futile Cycle

2b. Potential Conductance

2c. Potential Conductance

- In both 2c & 2b deprotonation of a proton that had been involved in the strong h-bond
- However the Ne2 proton is much more accessible the Nd1 and preliminary data suggests that the latter deprotonation is much less frequent.

Qin et al., JCPB, 2017
Third 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

- Cholesterol appears to facilitate viral budding through stabilizing the amphipathic helix in the membrane interface

- 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
How Many Lipids are Necessary to Ensure a Native Structure

Viral Exterior

40 lipids in van der Waals contact the M2 tetramer

Viral Interior

His-37 residues are the key to ion conductance

Paulino et al., in preparation
$^2$H Quadrupolar Powder Pattern Dynamics

CD$_3$ Alanine rotating about Ca-Cb axis & global rotation about bilayer normal

Effect of Methyl Axis Orientation wrt Bilayer Normal

Rotation Rate of Methyl Axis about Bilayer Normal

Paulino, PhD Dissertation, 2016
Lipid Content Influence on M2TM Global Rotation Diffusion Rate (DR)

M2TM d4Ala29
DOPC:DOPE 4:1 pH 7.5 @20 °C
Spectra Obtained at 19.4 T

1:80 → 1:30 P:L Ratio causes 50 fold reduction in Global Rotation

DR 9 x 10^5 s^-1

DR 2 x 10^4 s^-1

28 to 30 annular lipids surround M2TM

Paulino, et al., in preparation
Course Grain MD Simulations of M2TM Illustrate Oligomerization

- Permits simulation of 16 Tetramers in lipid bilayer
- Starting configurations – no tetramer-tetramer interactions

Xiadong Wang, Huan-Xiang Zhou
Oligomerization of the M2TM

- 2 Results from 16 simulations with 16 tetramers
- Using 1:28 molar ratio

Xiadong Wang, Huan-Xiang Zhou
Oligomerization of the M2TM

Cluster sizes distribution

- A dramatic difference upon increasing the lipid fraction from 1:28 to 1:80
- Explains the Andreas et al., dimer of dimer results

Xiadong Wang, Huan-Xiang Zhou
Take Home Messages

- More than Sparse Lipids are needed to Assure Native Structure
- A Tetramer does not mean Structural Uniformity
- Biology can be Exceptionally Heterogeneous
- Biology has Figured out how to Stabilize Charges in the Midst of the Lipid Bilayer

- Cholesterol binding to the amphipathic helix that stabilizes the pyramidal shape essential for viral budding.
- Solid State NMR is more than a Structural Biology Tool – It is a Tool for Functional Biology – the Characterization of Mechanisms and Mechanistic Rates