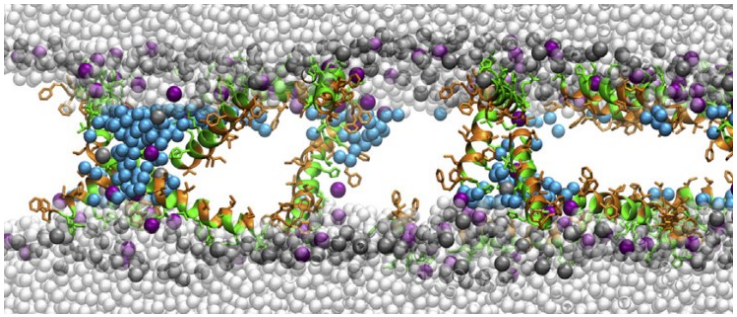
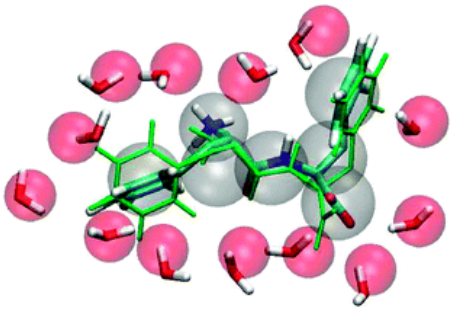
SOUSCC 2017

Characterization of an Anticancer Wasp Venom Component via Microsolvation Studies with Differential Mobility Spectrometry





Christian Ieritano, Steve Walker, W. Scott Hopkins*

March 18, 2017

(Perrin, Fu, Cotten, & Pastor, 2016)

Outlook

1. **Introduction**
   i. **Structure-activity relationships** of MP1 toxin from *Polybia Paulista*
   ii. The **drug discovery process**
   iii. Characterization of **MP1** structural motifs

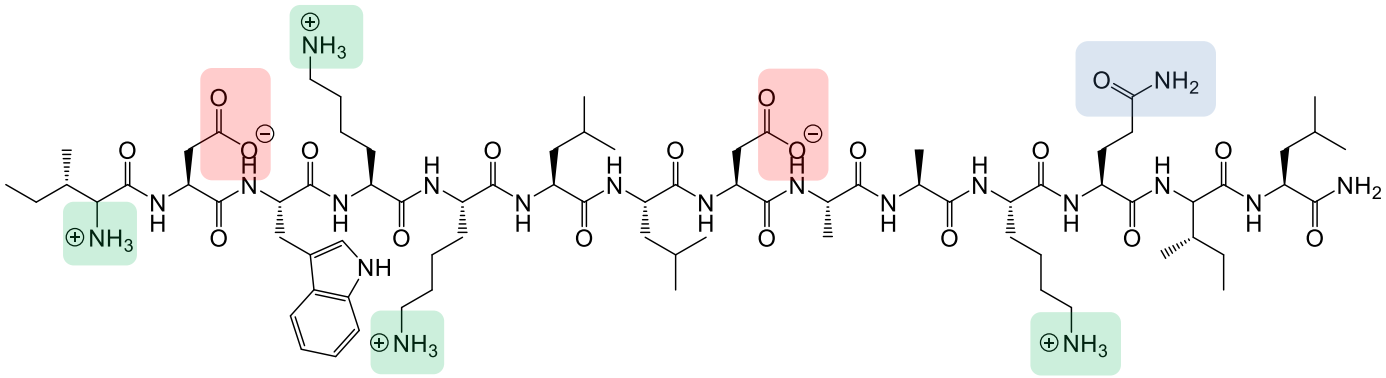
2. **Differential Mobility Spectrometry (DMS)**
   i. The **method** and its importance to **drug discovery**
   ii. Implication of DMS measurements on Polybia-MP1 structure and activity

3. **Microsolvation and Modelling**
   i. Bridging motifs may be the source of **selective activity**
   ii. Evolution of bulk, dynamic helical structure as a function of microsolvation

4. **Concluding Remarks and Future Directions**

Selectivity of MP1 Toxin for Bacterial Membranes

1. MP1 is a **cationic**, **amphipathic** helix which exists as part of the host's defense system



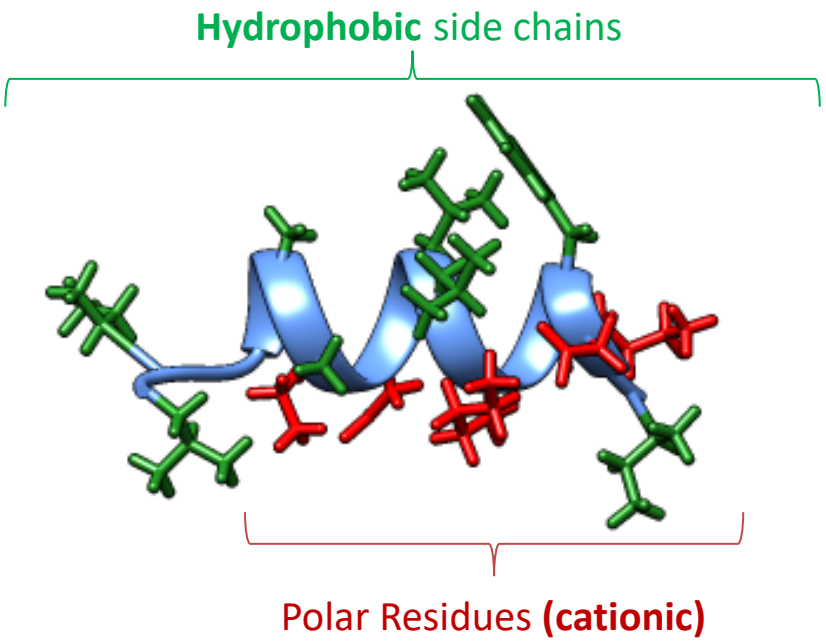
+2 charge at physiological pH

1655 Da

Polybia Paulista
(Brazilian wasp)

Polybia MP1 (**MP1**)

Wasp venom peptide



MP1 structure generated via iTASSER

Project Motivation

DRUG DISCOVERY

10,000 COMPOUNDS

PRECLINICAL

250 COMPOUNDS

CLINICAL TRIALS

5 COMPOUNDS

FDA REVIEW

1 FDA APPROVED DRUG

~6.5 YEARS

~7 YEARS

~1.5 YEARS

Project Motivation

MP1 has exhibited:

- Selective activity against bladder and prostate cancer cell lines
- No cytotoxicity to rat host models
- Facile poration to malignant/bacterial tissue mimetics

DRUG DISCOVERY

10,000 COMPOUNDS

PRECLINICAL

250 COMPOUNDS

CLINICAL TRIALS

5 COMPOUNDS

FDA REVIEW

1 FDA APPROVED DRUG

MP1 currently in **pre-clinical** stages of development

~6.5 YEARS | **~7 YEARS** | **~1.5 YEARS**

Project Motivation

However…..

A lack of structural characterization have ultimately hindered the implementation of MP1 to human trials.

To date, only **one molecular dynamics (MD)** simulation (in non-biological conditions) has been conducted.

No solid-state NMR characterization of peptide-lipid interaction

No MD of **MP1 interactions with lipid bilayers**

No crystal structure

To remedy this:

Use **Differential Mobility Spectrometry (DMS)** as a probe for:

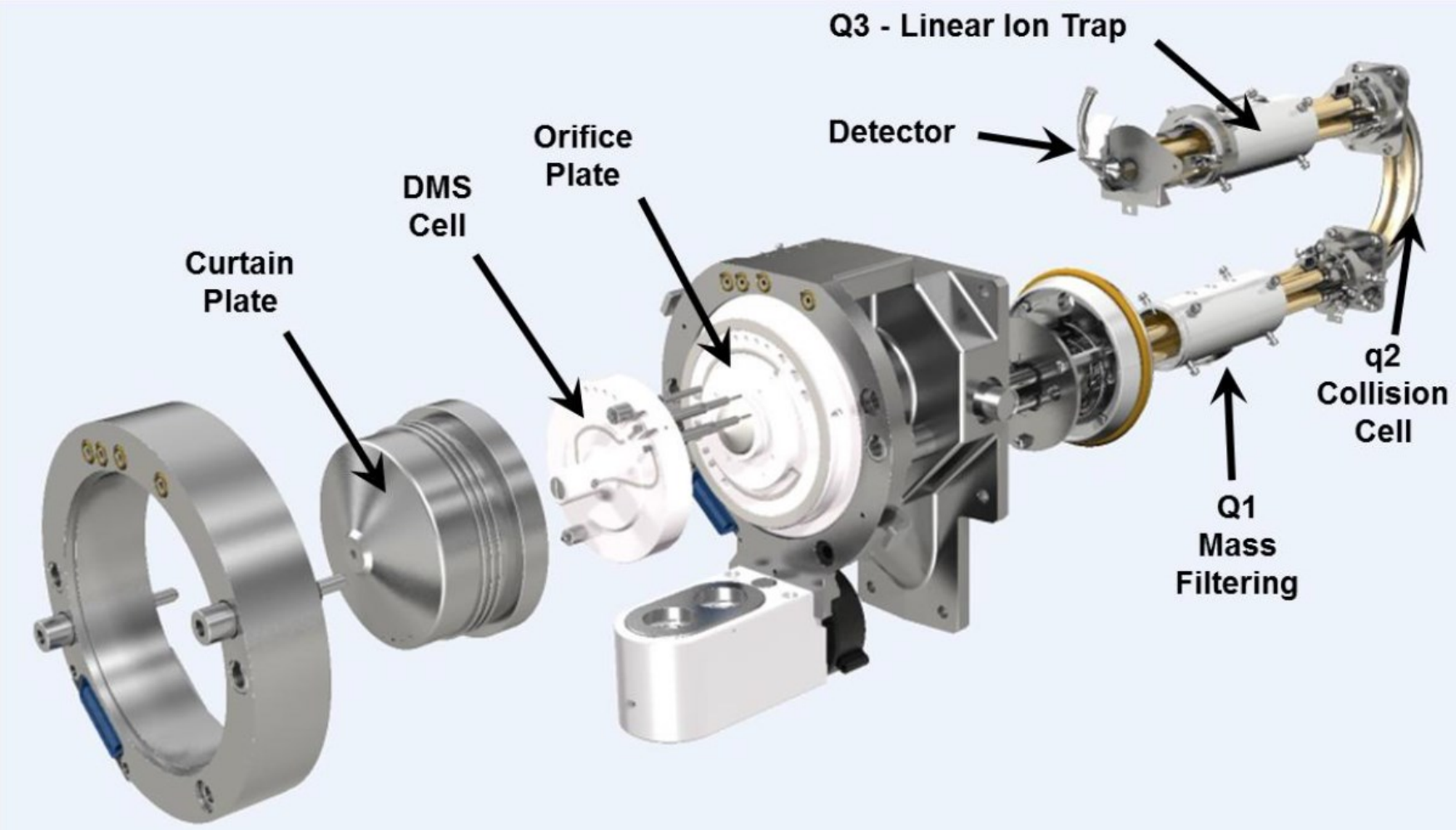
- Structure
- Physicochemical Properties

Utilize computational **microsolvation studies** to:

- Monitor evolution of solution phase structure
- Explore the PES of MP1
- Infer **structure activity relationships** from calculated geometries

Differential Mobility Spectrometry as a Structural Probe

How can we use Differential Mobility Spectrometry to characterize MP1?



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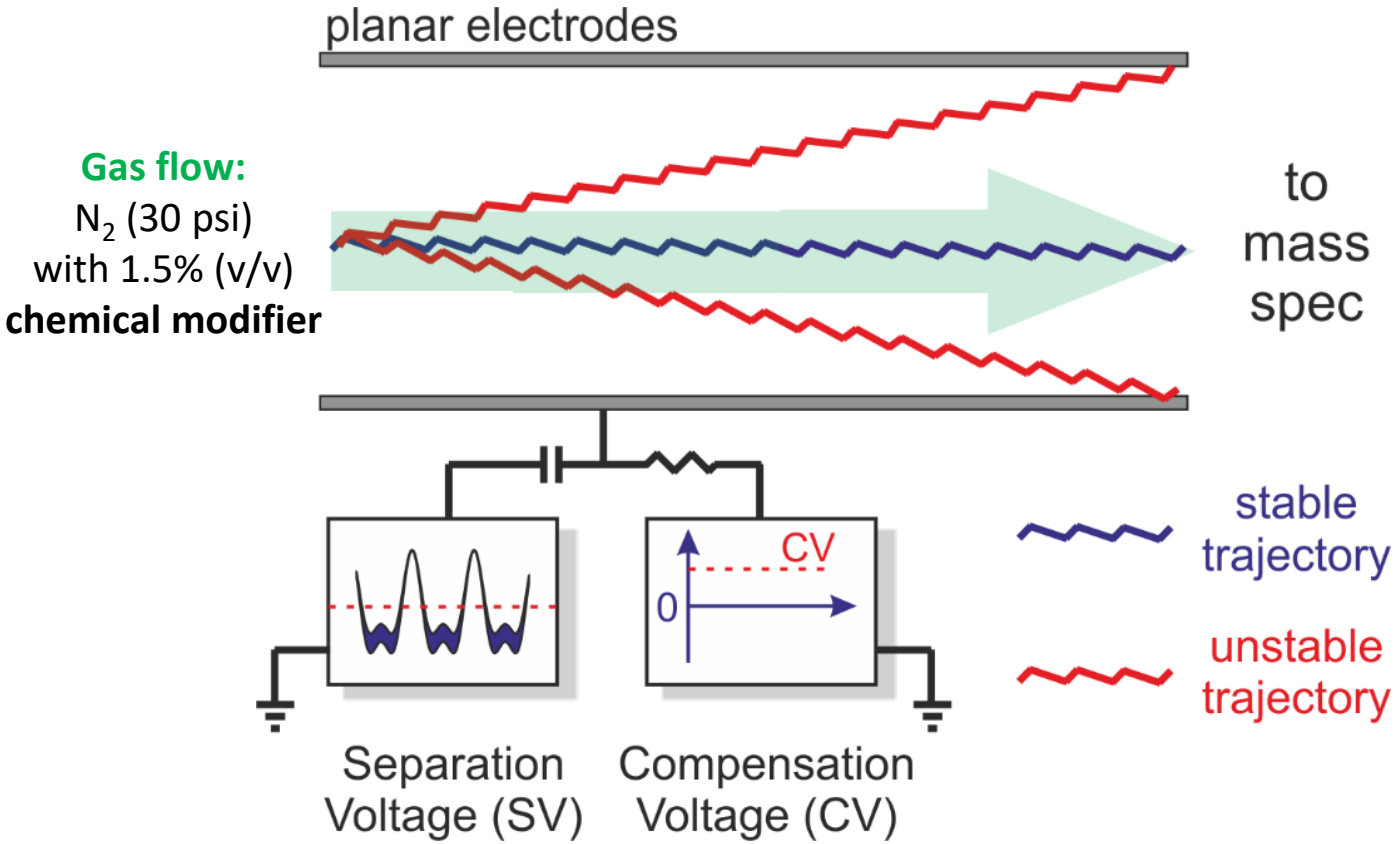
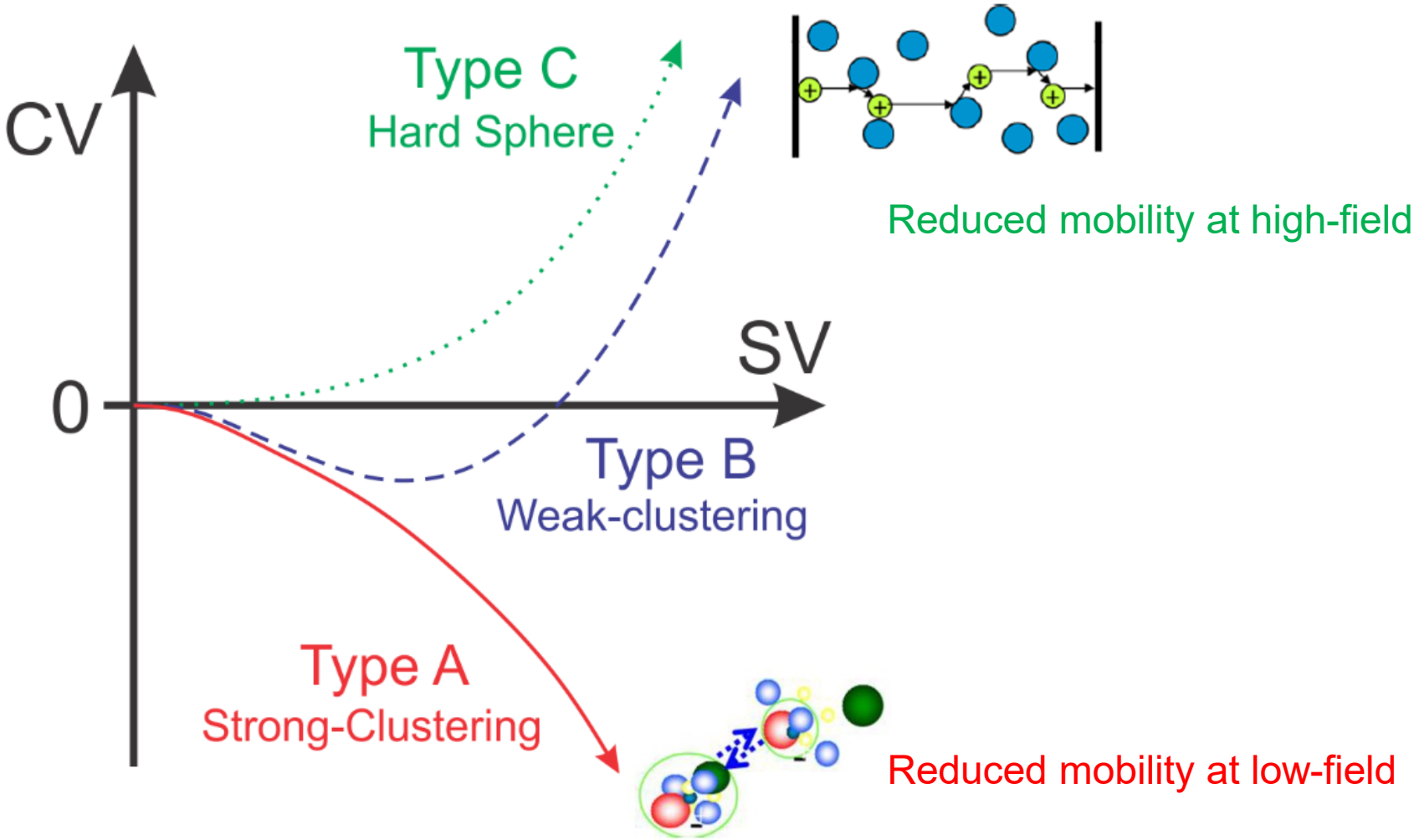


The magnitude of the **compensation voltage** (CV) is indicative of the strength **ion-solvent interactions.**

Hopkins, W. S.. *Mol. Phys.* 113, 3151 - 3158

Differential Mobility Spectrometry as a Structural Probe

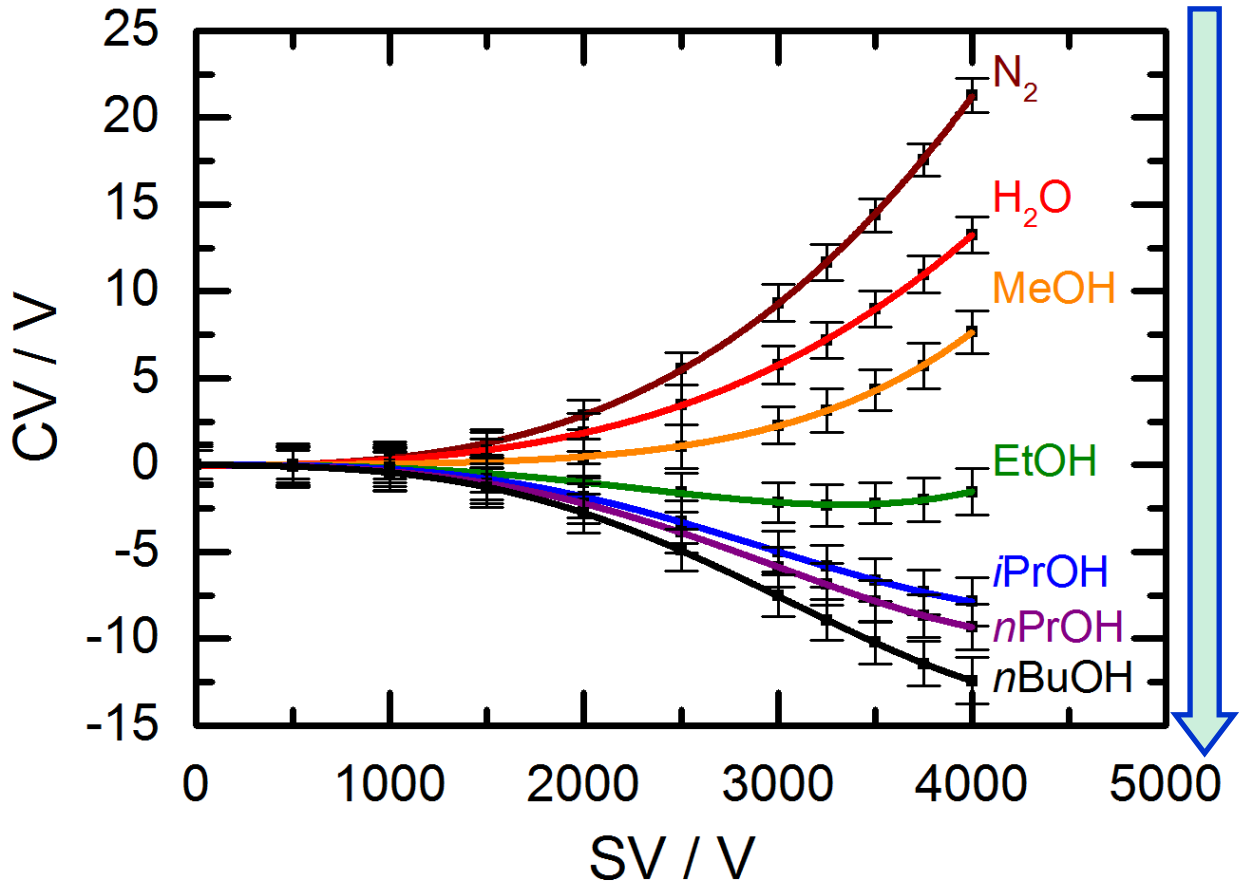
How can we use Differential Mobility Spectrometry to characterize MP1?



Hopkins, W. S. *Mol. Phys.* 113, 3151 - 3158

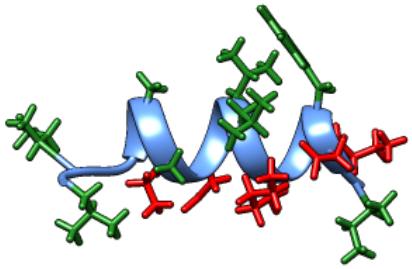
Dispersion Plots Indicate Ion-Solvent Interactions with Protic Modifiers

MP1 m/z 828 $[M + 2H]^{2+}$



Increasing alcohol chain length

Hydrophobic Residues



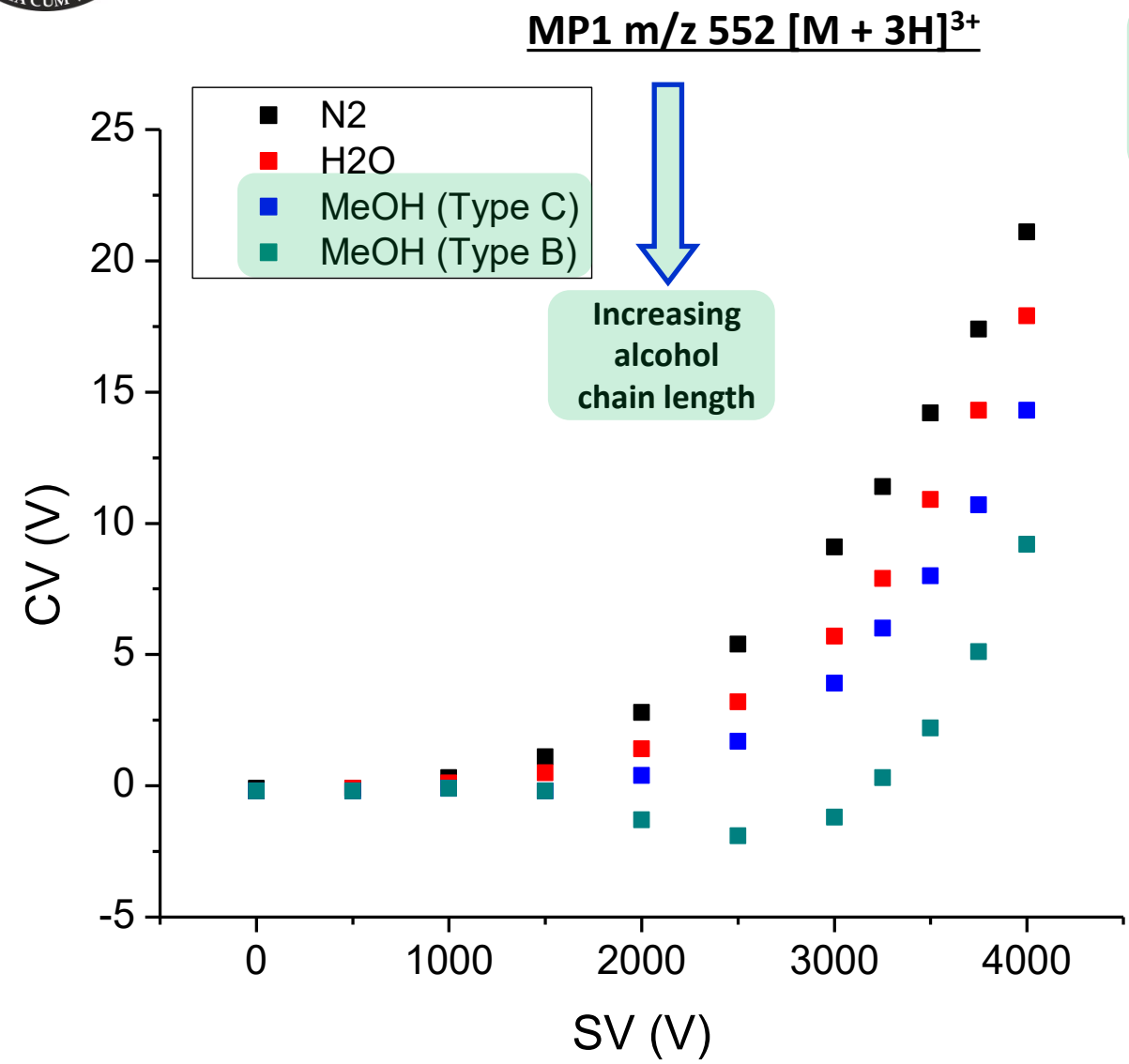
Cationic Residues

Increasing ion-solvent clustering interactions with longer chain alcohols

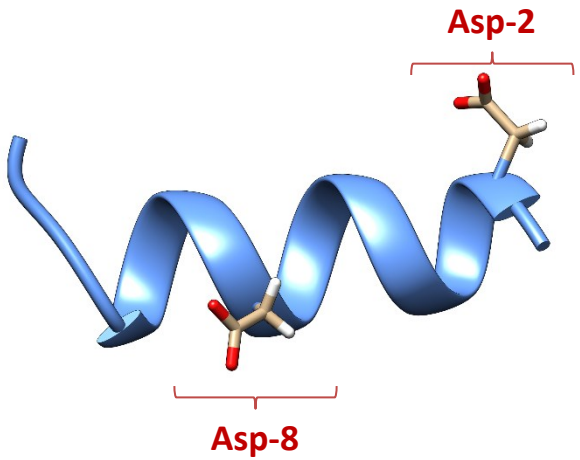
Non-polar, protic modifiers **induce helical geometry**

Helical geometry would **maximize** ion-solvent interaction (more later)

Dispersion Plots Indicate Ion-Solvent Interactions with Protic Modifiers



Using a MeOH modifier, isomers corresponding to **uniquely protonated Asp residues** emerge

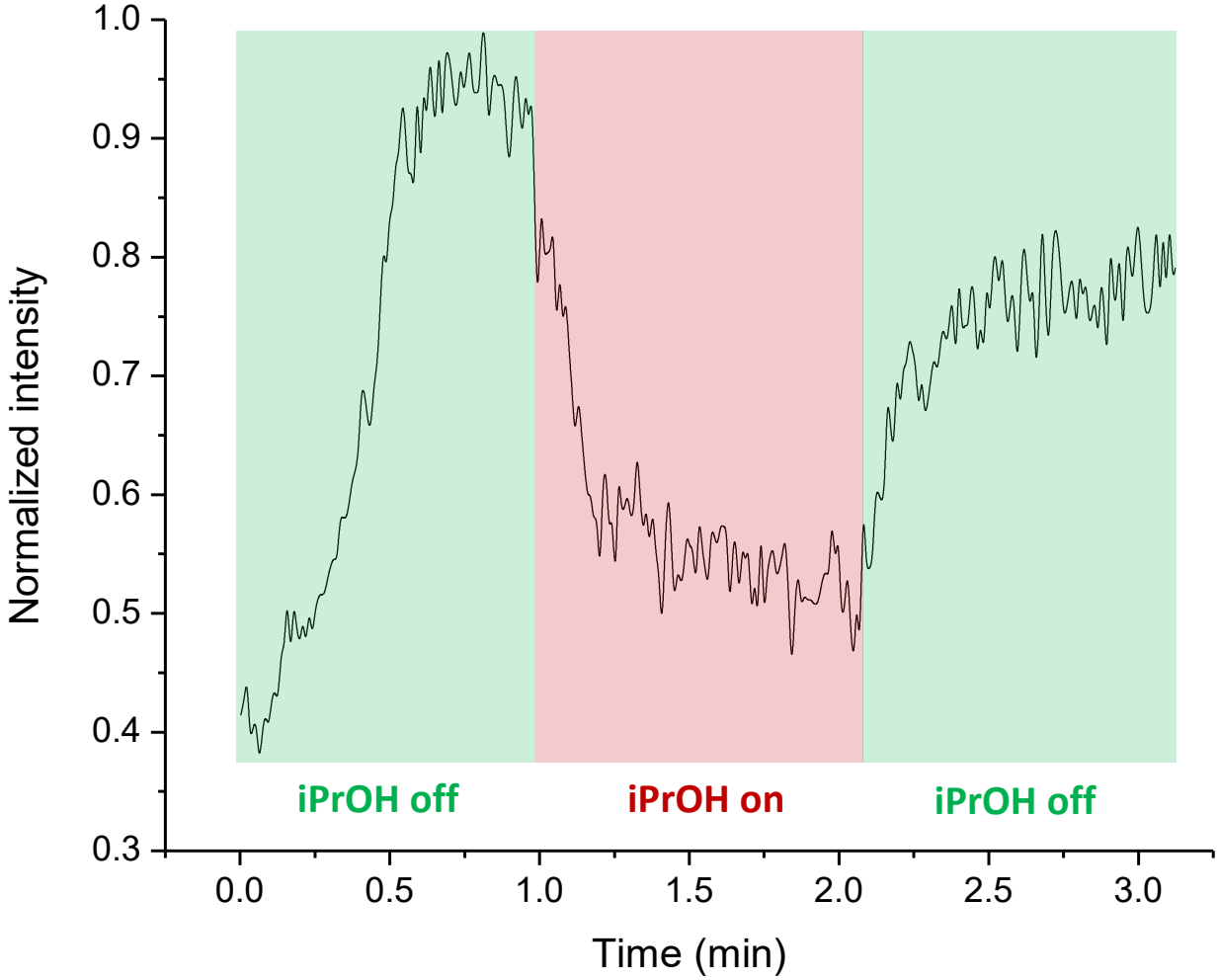


Huge depletion of m/z 552 signal intensity with MeOH modifier vs. H_2O mod (data not shown)

No m/z 552 signal observed with EtOH modifier and longer alcohols

Dispersion Plots Indicate Ion-Solvent Interactions with Protic Modifiers

Ion intensity (m/z 552) in the **presence** and **absence** of iPrOH modifier



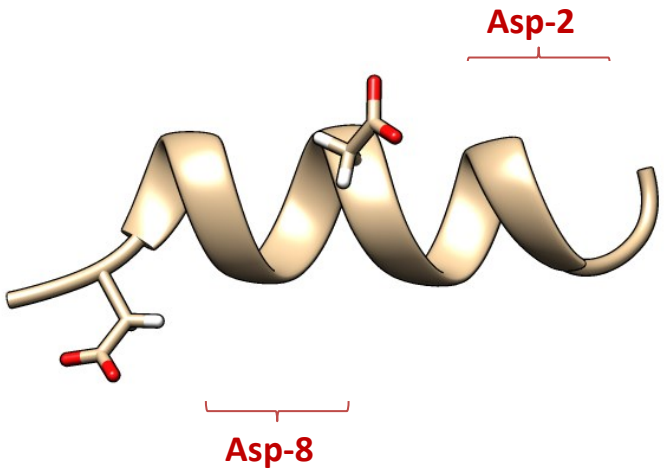
Introduction of modifier **abstracts** a proton from **MP1 Asp residue**

Yields insight into acidity of Asp residues

Allows for dynamic visualization of proton abstraction by modifier

Proton Abstraction Correlates with Gas-Phase Proton Affinities

Species	Gas-Phase Proton Affinity (kJ mol^{-1})
H_2O	691.0
MeOH	754.3
EtOH	776.4
MeCN	779.2
*n*PrOH	786.5
*n*BuOH	789.2
*i*PrOH	793.0
Acetone	812.0



Abstraction of Asp proton supresses m/z 552

Proton Abstraction Correlates with Gas-Phase Proton Affinities

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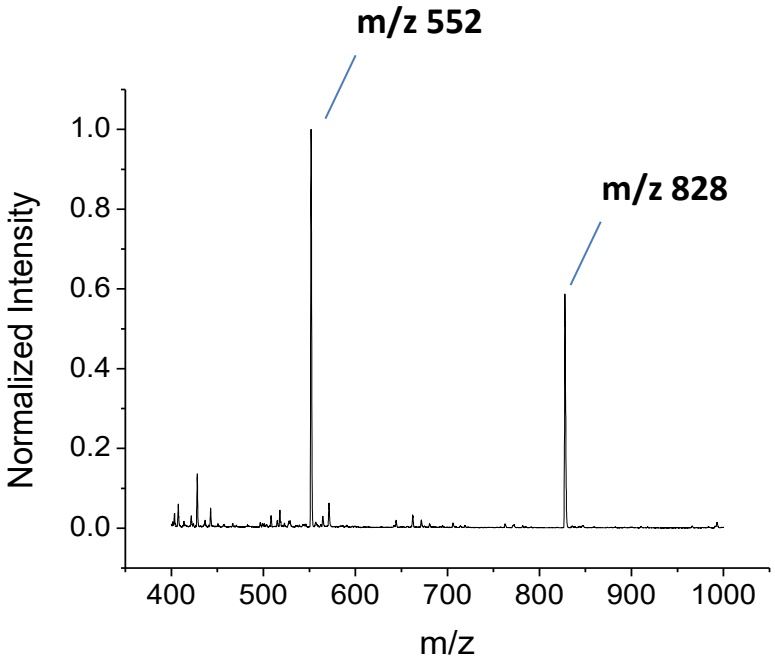
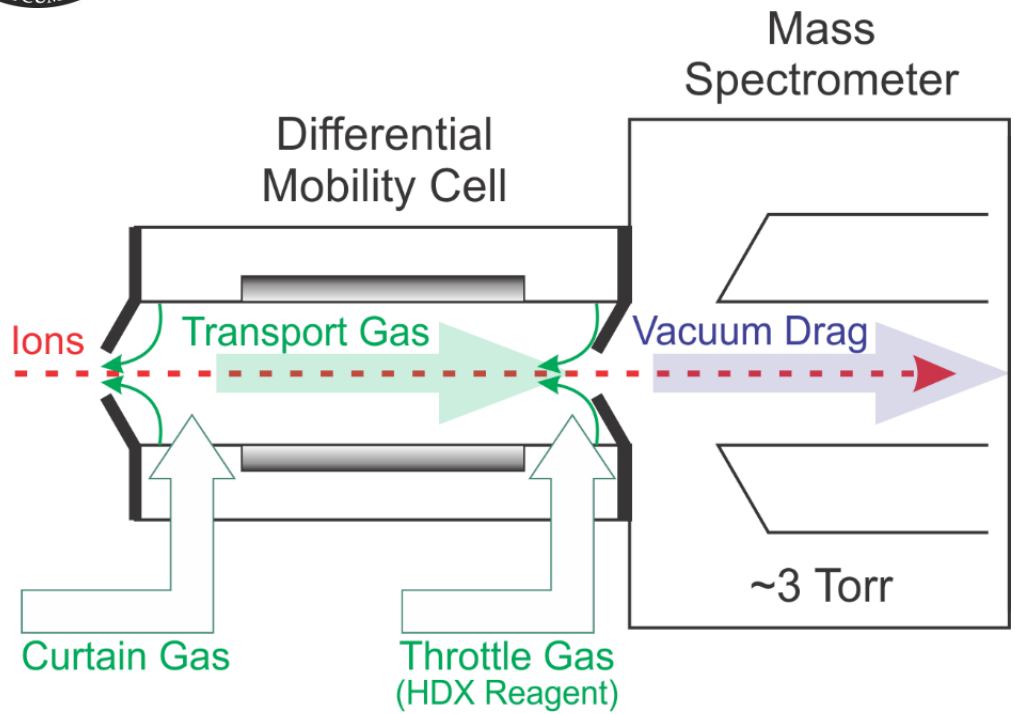


Observation likely a combination of:

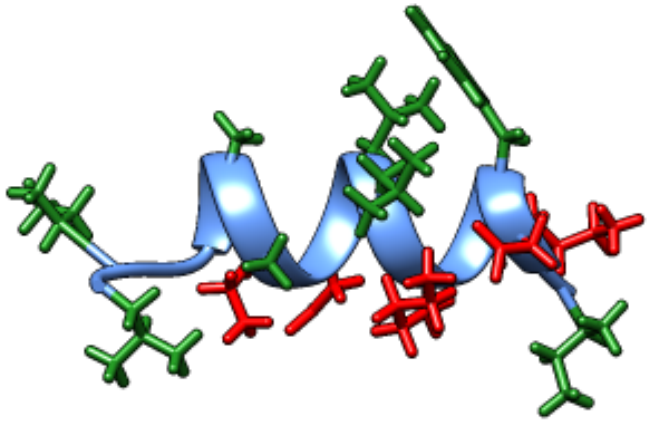
- Aprotic modifier induced unfolding
- Proton affinity variations between monomeric and clustered modifier species
- Proton abstraction through hydrogen bound network of solvent

DMS-HDX also Indicates Helical MP1 Structure



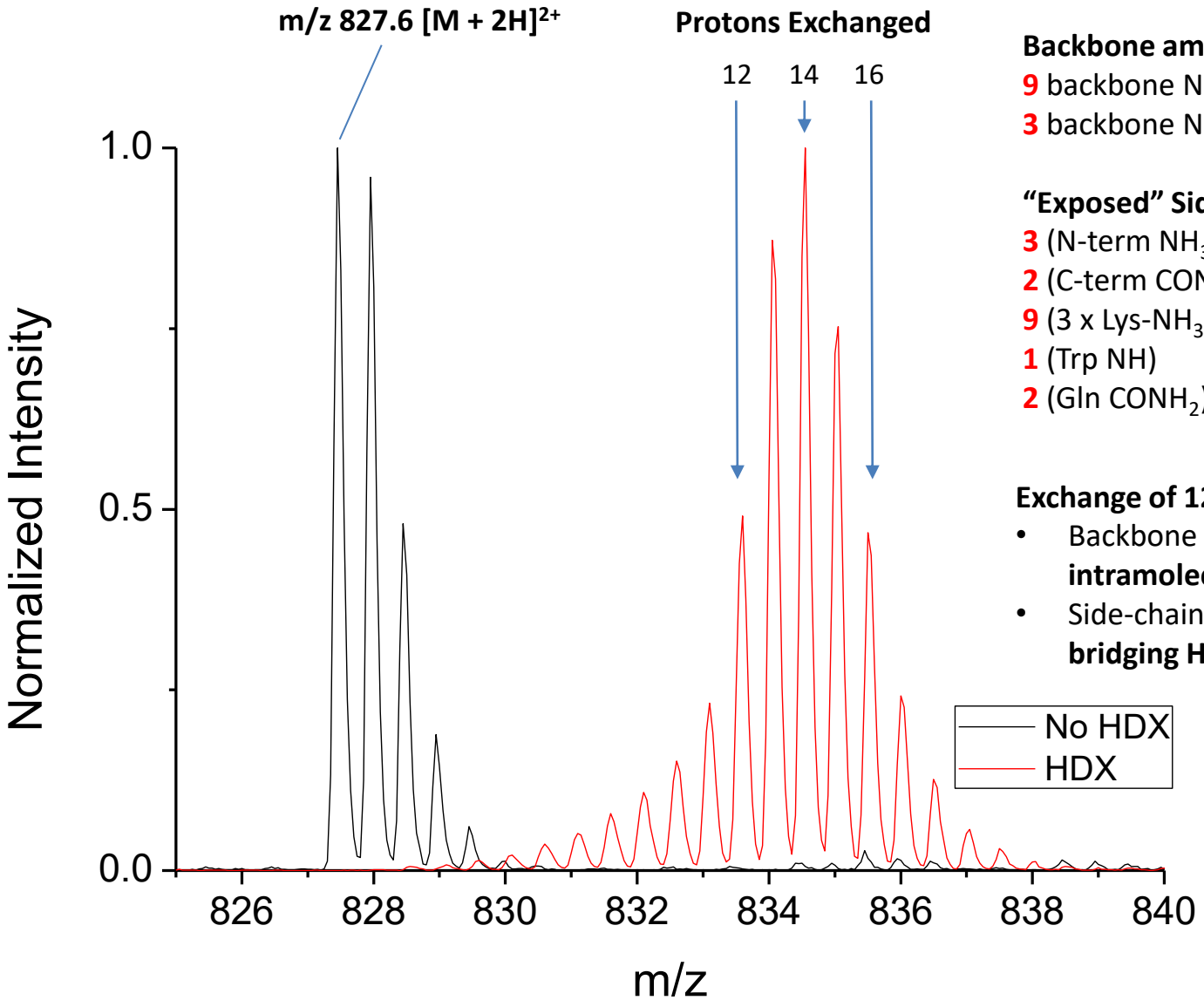
Introduce Hydrogen-Deuterium exchange (HDX) reagent (i.e. **D_2O**) to DMS/MS interface

Allows for selective exchange of **exposed**, exchangeable protons. e.g.
NH_2 (Lys, N-terminus)
$CONH_2$ (Gln, backbone amide, 'C-terminus')



Zhu, S., *et al*.. *J. Am. Soc. Mass Spectrom.*, 27, 991 - 999

DMS-HDX also Indicates Helical, Bridged MP1 Structure



Exchangeable Protons

Backbone amide:
9 backbone NH (helix)
3 backbone NH (coil)

12

"Exposed" Side Chain
3 (N-term NH_3)
2 (C-term $CONH_2$)
9 (3 x Lys-NH_3)
1 (Trp NH)
2 (Gln $CONH_2$)

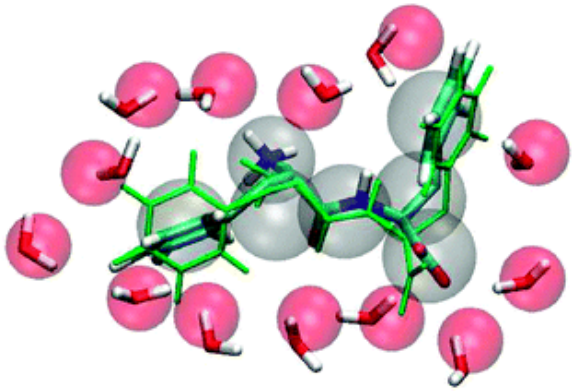
17

Total: 29

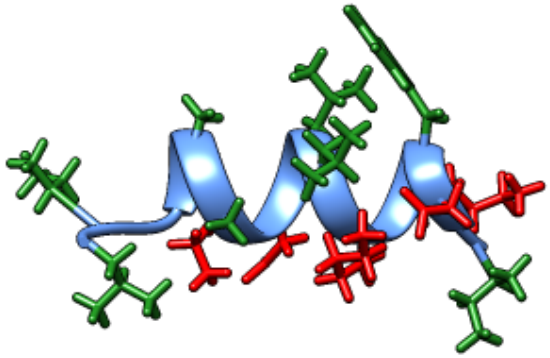
Exchange of 12-16 protons indicate:

- Backbone amide NH locked in **intramolecular** H-bond (**α-helix**)
- Side-chain residues involved in **bridging H-bonded interactions**

Computational Modelling and Microsolvation Studies on a Monster Peptide



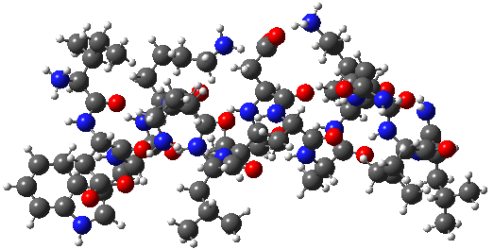
Computational Approach to **Modelling**

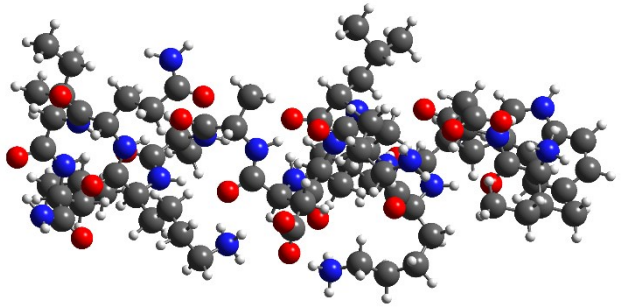


Input geometry based on previous MD-simulation

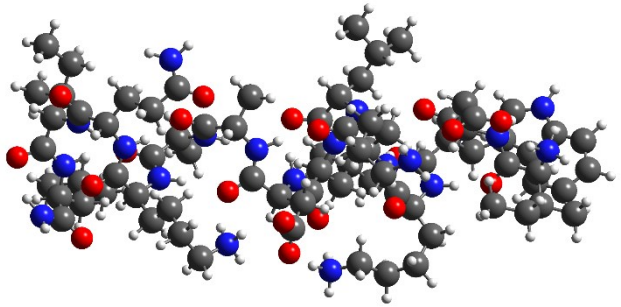
BH routine to low-energy identify side-chain configurations

Targeted Potential Energy Surface Mapping



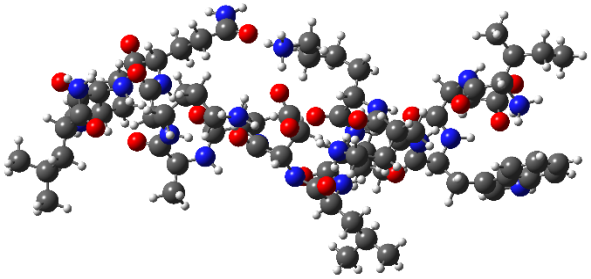


Unique Isomer refinement with Density Functional Based Tight-Binding (DFTB)

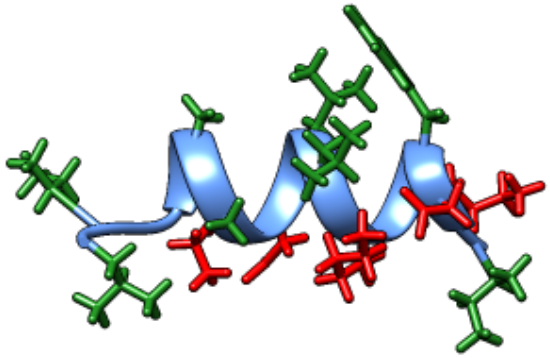


Optimization with Density Functional Theory (DFT)

B3LYP/6-31G with Empirical Dispersion correction (GD3)



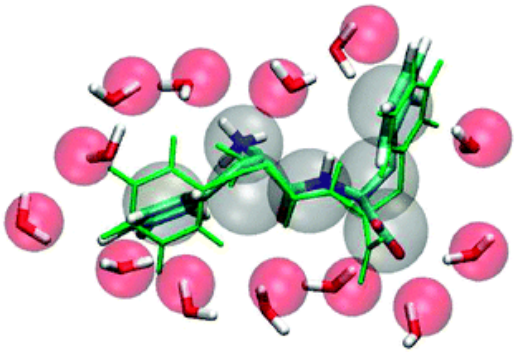
Computational Approach to **Microsolvation**

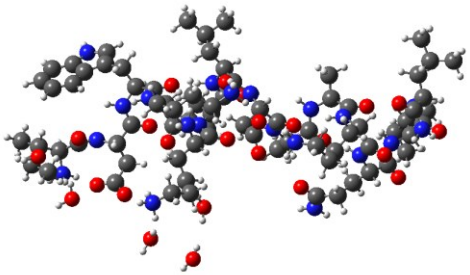


Input geometry based on previous BH routine

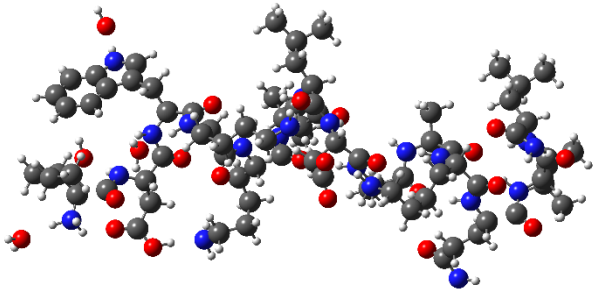
BH routine to $(H_2O)_n$ binding pockets (n = 1- 15)

Targeted Potential Energy Surface Mapping





Unique isomer refinement with Density Functional Based Tight-Binding (DFTB)



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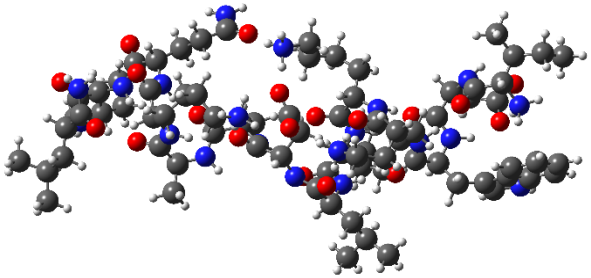
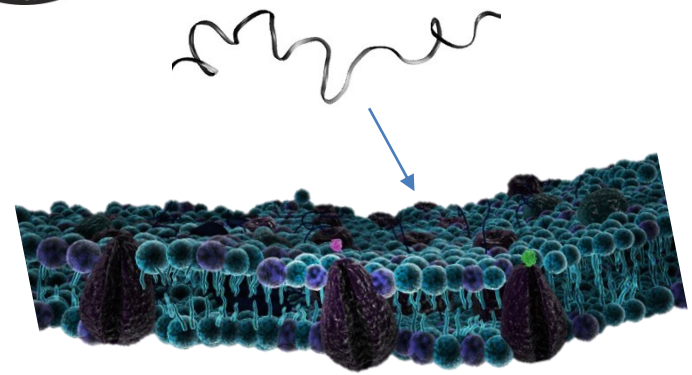
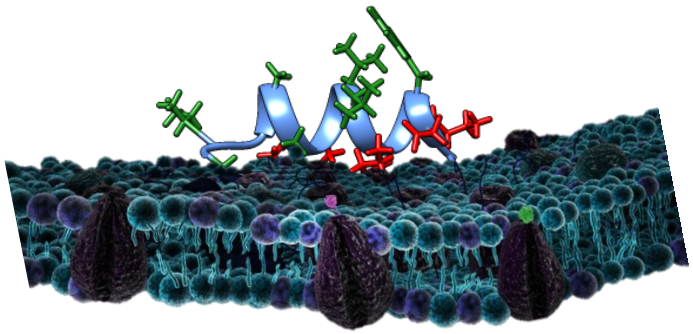


Structural Implications of Microsolvation



MP1 binding to tumor membrane



Surface Unbound State (Dynamic Coil)

Surface Bound State (Folded Helix)

Bulk Solvation Profile

- Highly aqueous environment
- Competition between hydrogen bonding between backbone and solvent
- Interchanging hydrogen bonds leads to dynamic, coil motif

Microsolvation Implications (hypothesis)

- Higher degrees of microsolvation should induce reduced helicity

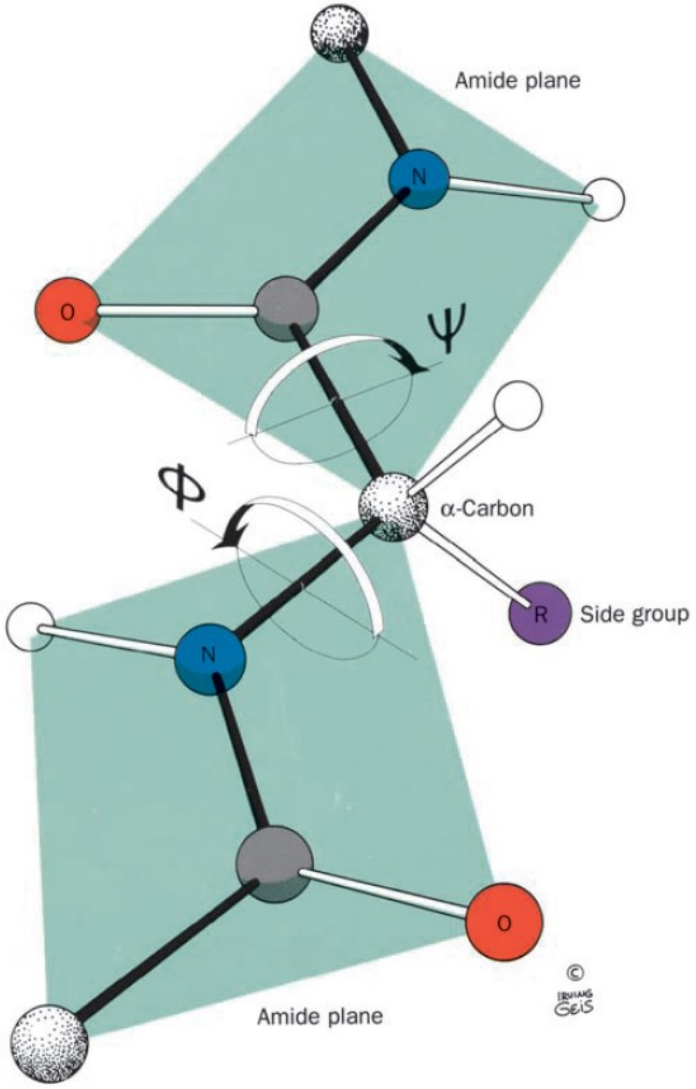
Bulk Solvation Profile

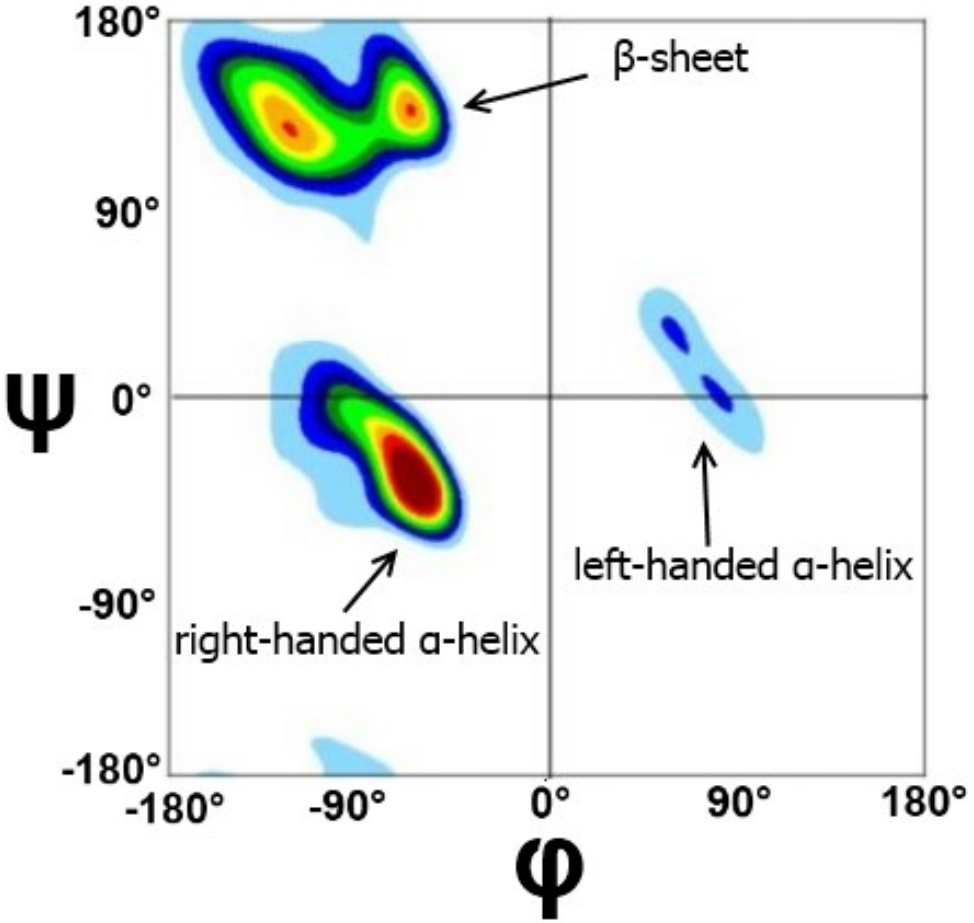
- Membrane binding and insertion into lipid core yields a highly non-polar environment
- High degree of backbone hydrogen bonding
- Decrease in favourability of unfolding leads to helical geometry

Microsolvation Implications (hypothesis)

- Purely gas-phase structures should be predominantly helical

Quantitatively Assessing Helicity – Ramachandran Plots

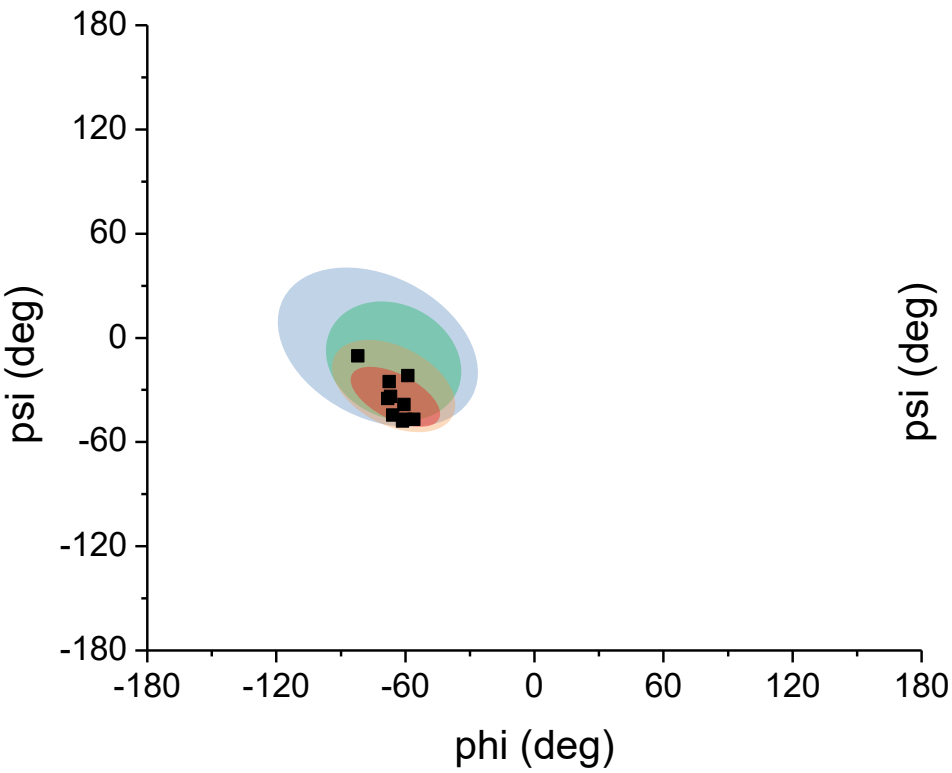




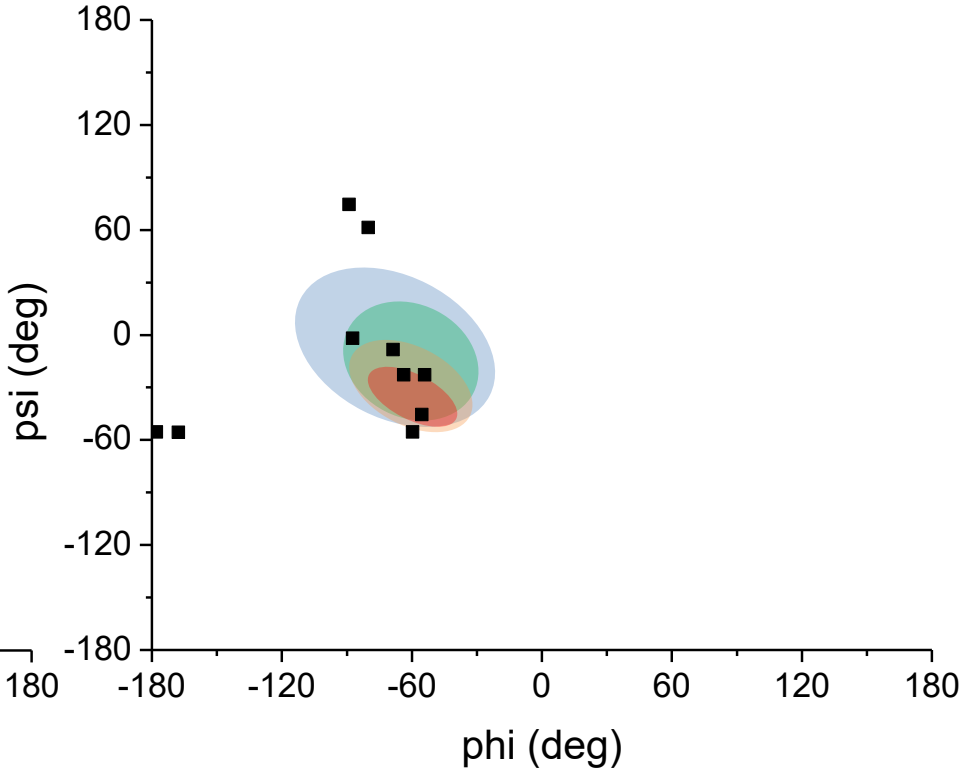
Voet & Voet. Biochemistry (4th Ed.), Hovmoller *et al*. *Acta Cryst.* D, 58, 768 - 776

Quantitatively Assessing Helicity – Ramachandran Plots

Bare MP1



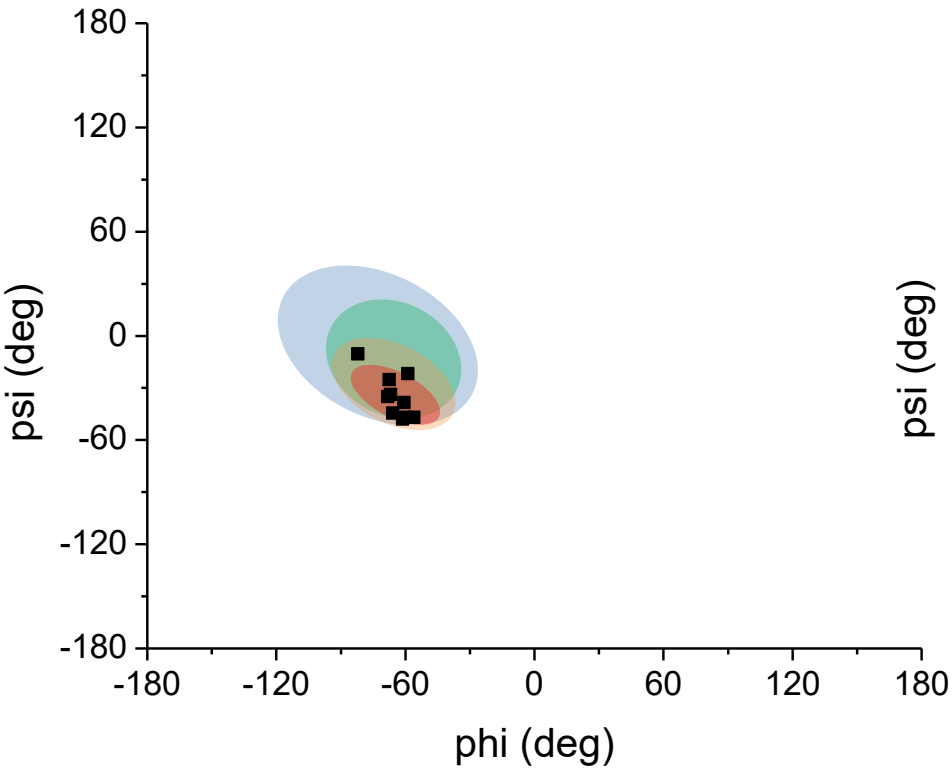
MP1·H_2O



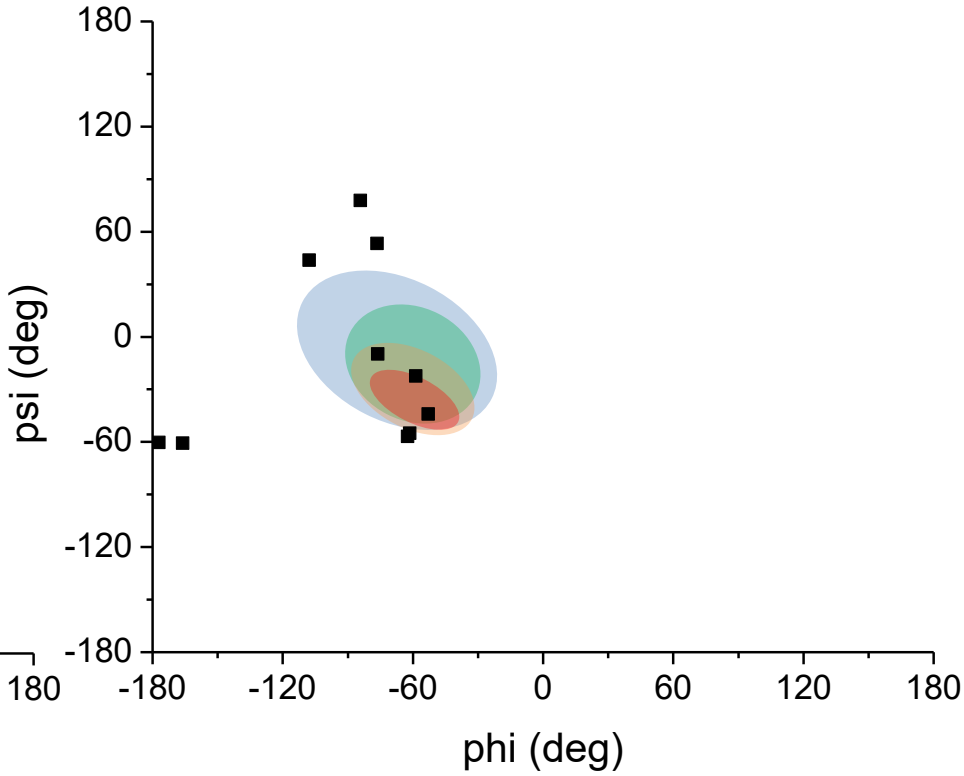
1 water

Quantitatively Assessing Helicity – Ramachandran Plots

Bare MP1



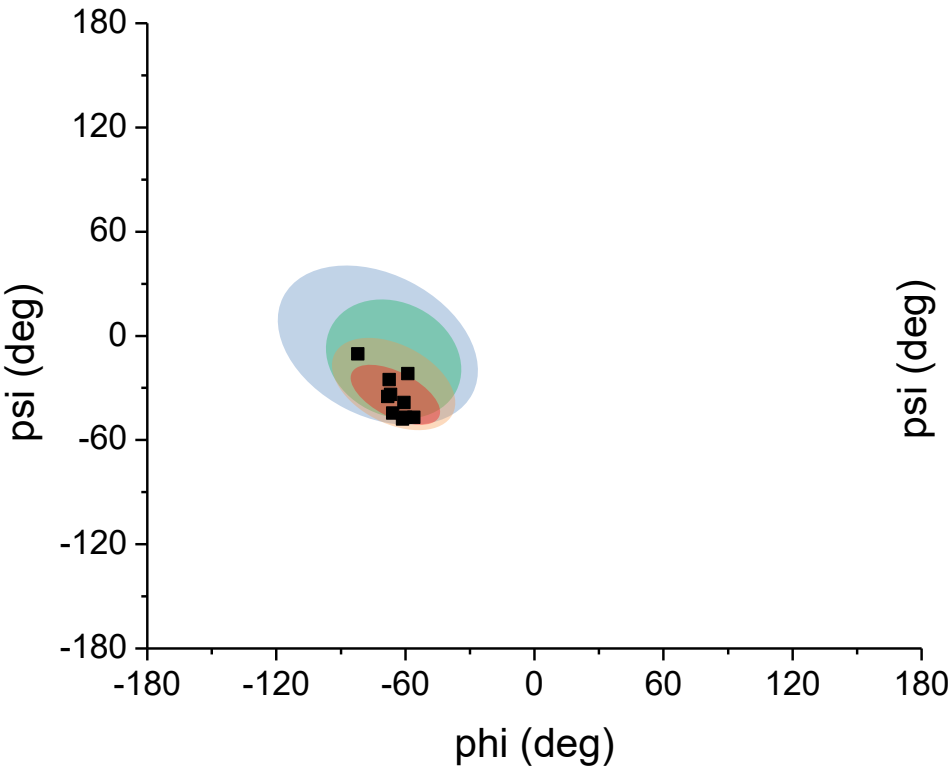
MP1·$(H_2O)_2$



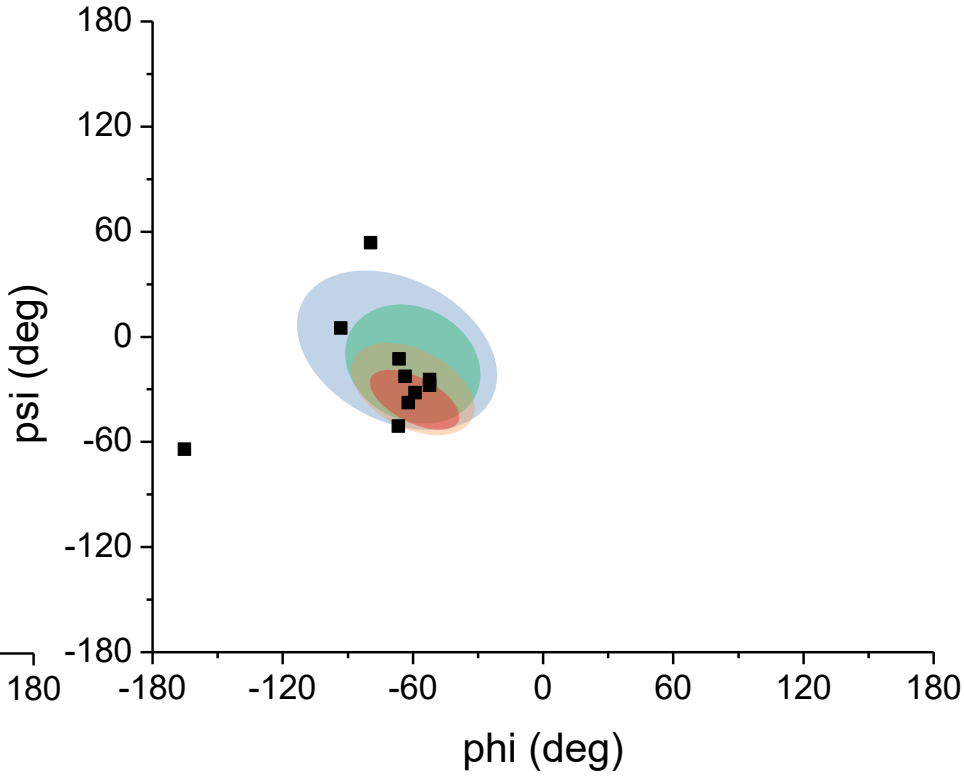
2 water

Quantitatively Assessing Helicity – Ramachandran Plots

Bare MP1



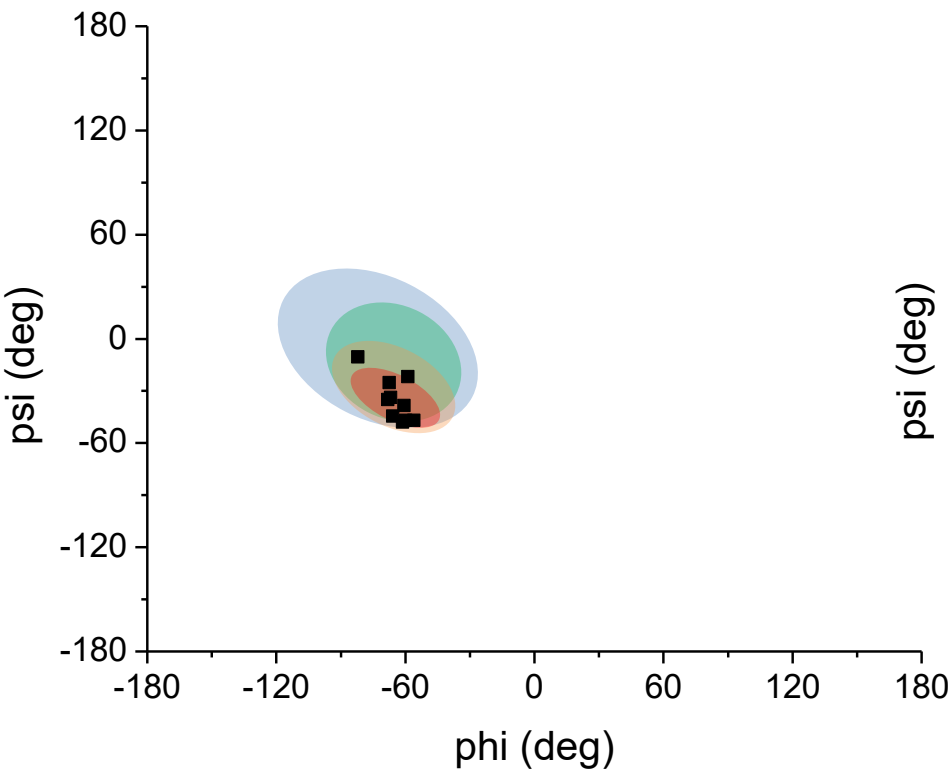
MP1·$(H_2O)_3$



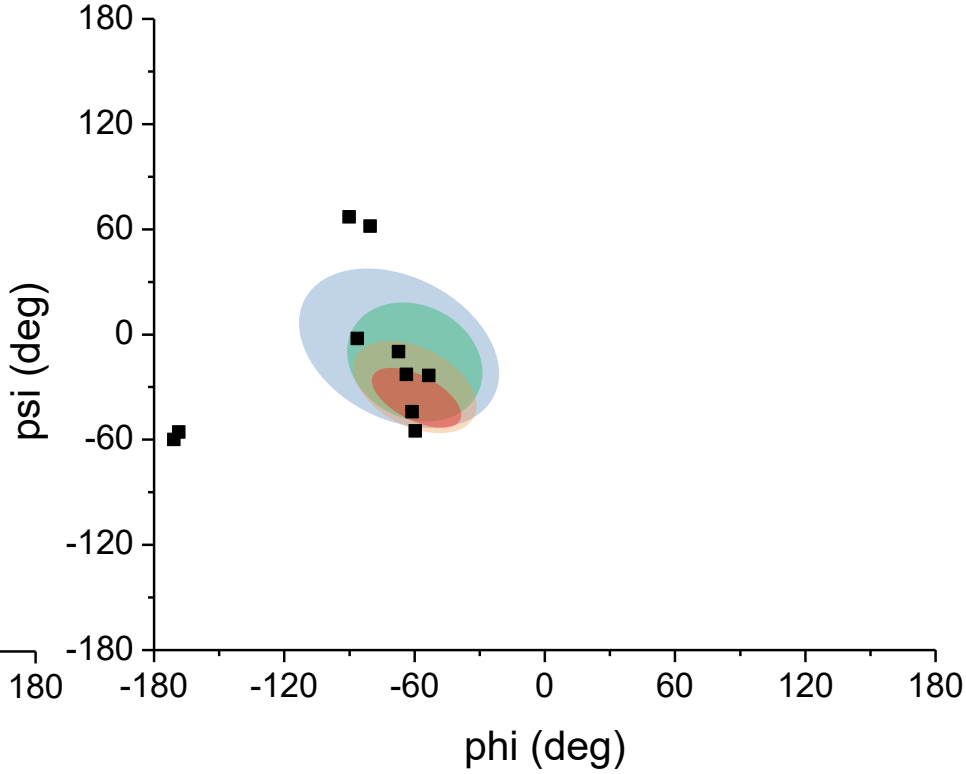
3 water

Quantitatively Assessing Helicity – Ramachandran Plots

Bare MP1



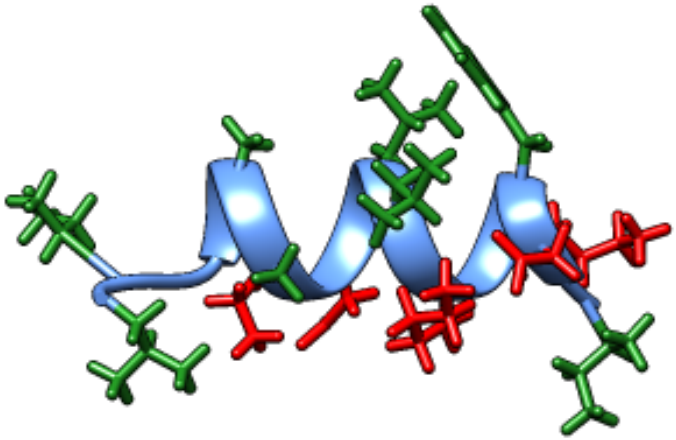
$MP1\cdot(H_2O)_4$



Decreasing helicity with increasing solvation

4 water

Concluding Remarks, Future Directions, and Acknowledgements



DMS measurements:

- Corroborate helical nature of MP1 (HDX exchange)
- Provide insight to the gas phase acidity of MP1

Microsolvation Studies:

- Calculated geometries exhibit bridging interactions between Lys and Asp side chains
- Increased solvation favours partial unfolding. Gas phase measurements could indicate MP1 conformation in membrane core

Future Work (Continuation into M. Sc.)

- Conduct DMS/HDX experiments using Time Resolved Electrospray Ionization (TRESI)
- Monitor proton abstraction at varying modifier compositions
- Polish up final DFT calculations and making legible figures

Acknowledgements

compute • calcul CANADA

SHARCNET™

NSERC CRSNG

Pfizer

SCIEX

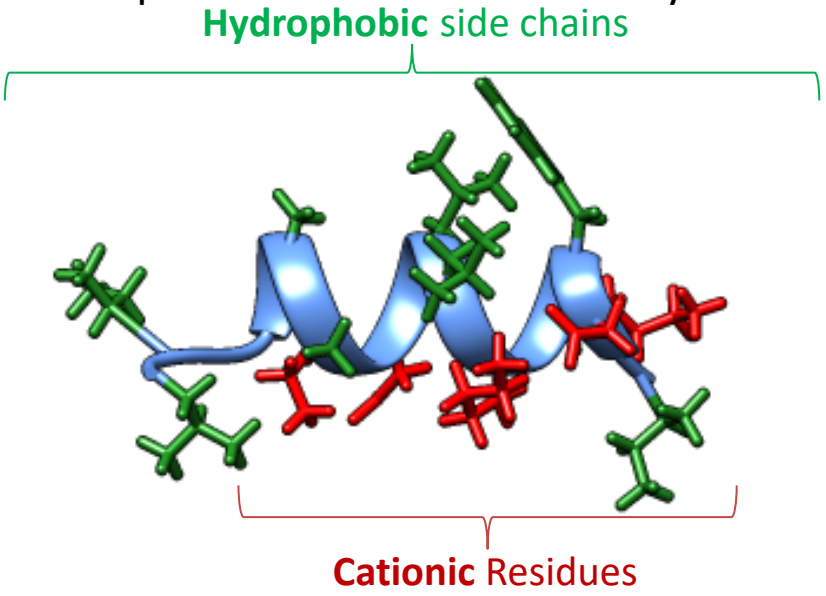
Josh Featherstone
Dr. Liz Meiering
Dr. Thorsten Diekemann

Selectivity of MP1 Toxin for Bacterial Membranes

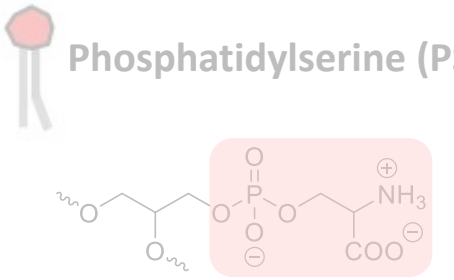
1. MP1 is a **cationic**, **amphipathic** helix which exists as part of the host's defense system

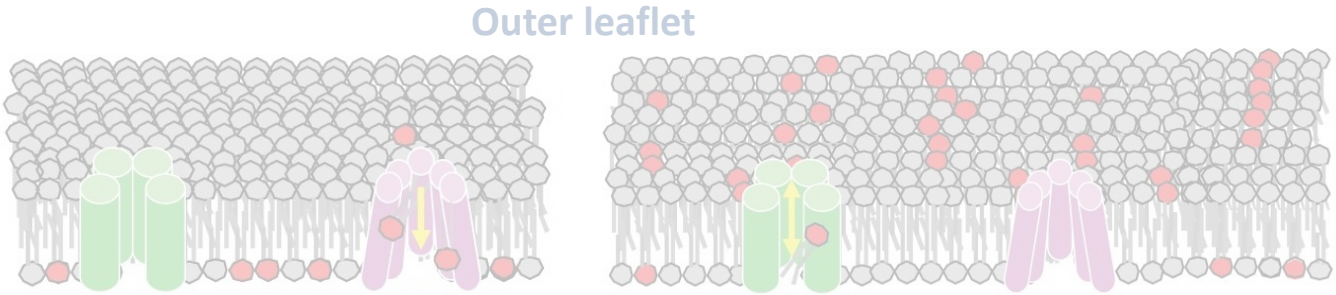
Polybia Paulista
(Brazilian wasp)

Polybia MP1 (**MP1**)
Wasp venom peptide



2. Externalization of **anionic (negative)** phospholipid head groups drive selective action





Host (healthy) cell
Asymmetric, **neutral** outer leaflet maintained by PS specific transferases

Bacterial/Malignant Cell
Anionic outer leaflet
No/defective PS transferase

(MP1 Structure Generated via iTASSER, Huang, Feng, Yan, Hao, & Chen, 2015)

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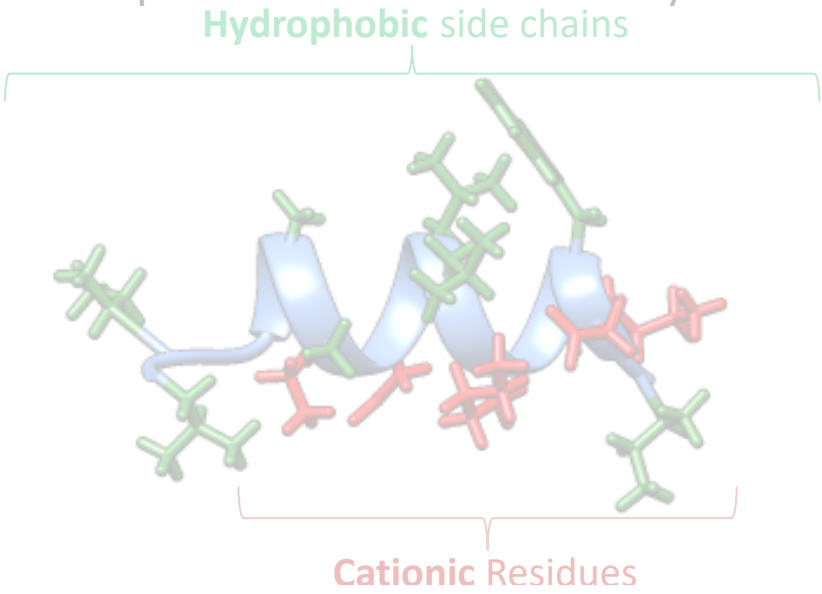

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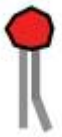
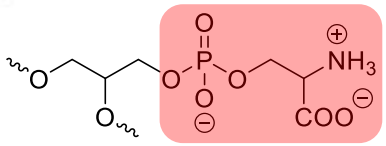
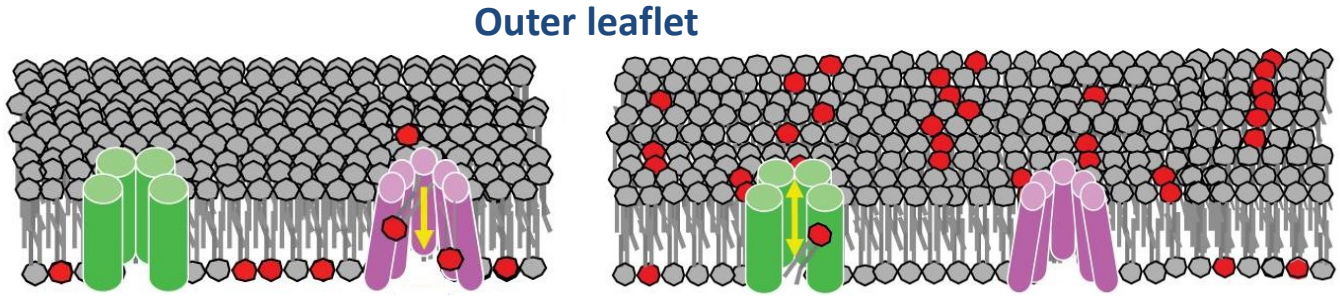


Phosphatidylserine (PS)





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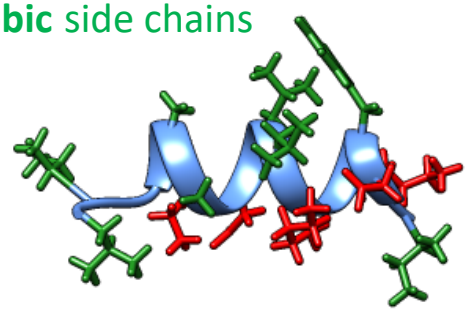
Selectivity of MP1 Toxin for Bacterial Membranes

Hydrophobic side chains

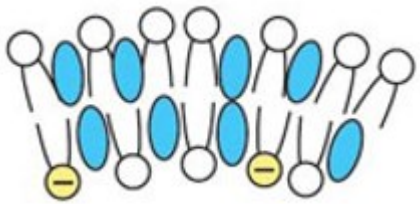


Cationic Residues

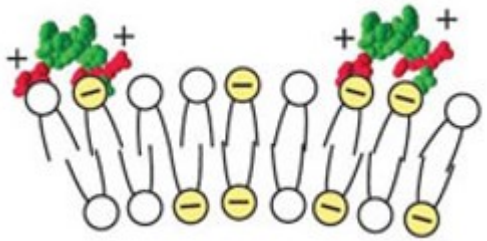
Typical, host cell membrane

Bacterial Cytoplasmic membrane

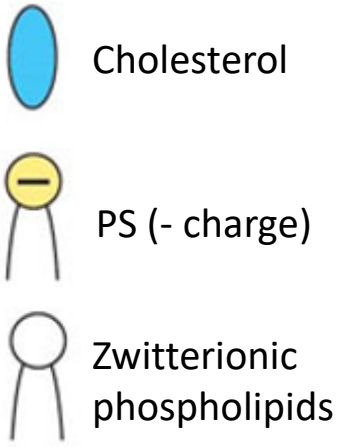
Outer leaflet



No interaction. No driving force to bind



Electrostatics drive peptide-lipid binding



Leads to **membrane poration** and **cell death** (by depolarization)

Hopkins, W. S.. *Mol. Phys.* 113, 3151 - 3158

Selectivity of MP1 Toxin for Bacterial Membranes

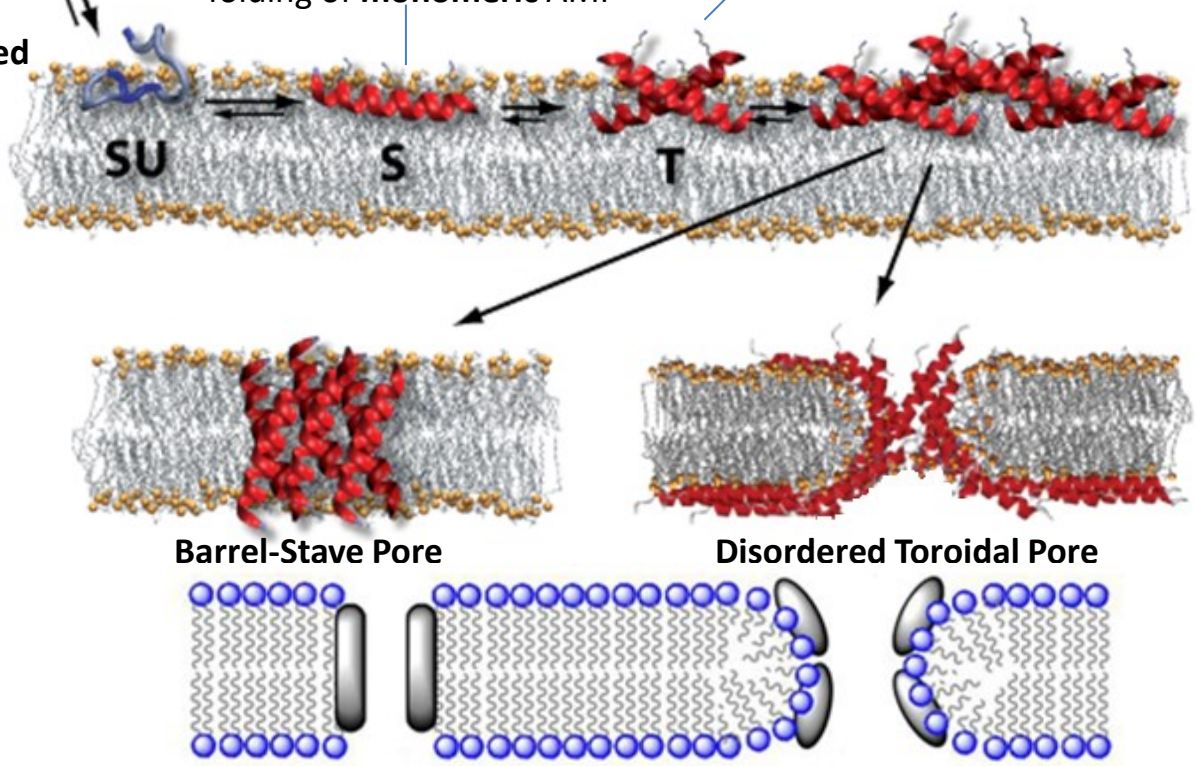
A general consensus from multiple MD simulations indicate a possible mechanism:

Unfolded, dynamic AMP

S-State
Energetically favoured helical folding of **monomeric** AMP

T-State
Combination of S-states (from exposed hydrophobics) drives aggregation and further membrane penetration

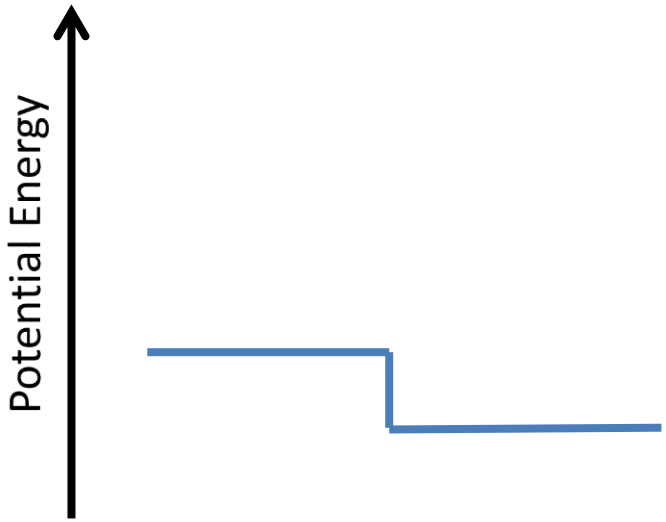
Surface, unfolded (SU)
AMP loosely bound to membrane via 'carpet bomb' exposure



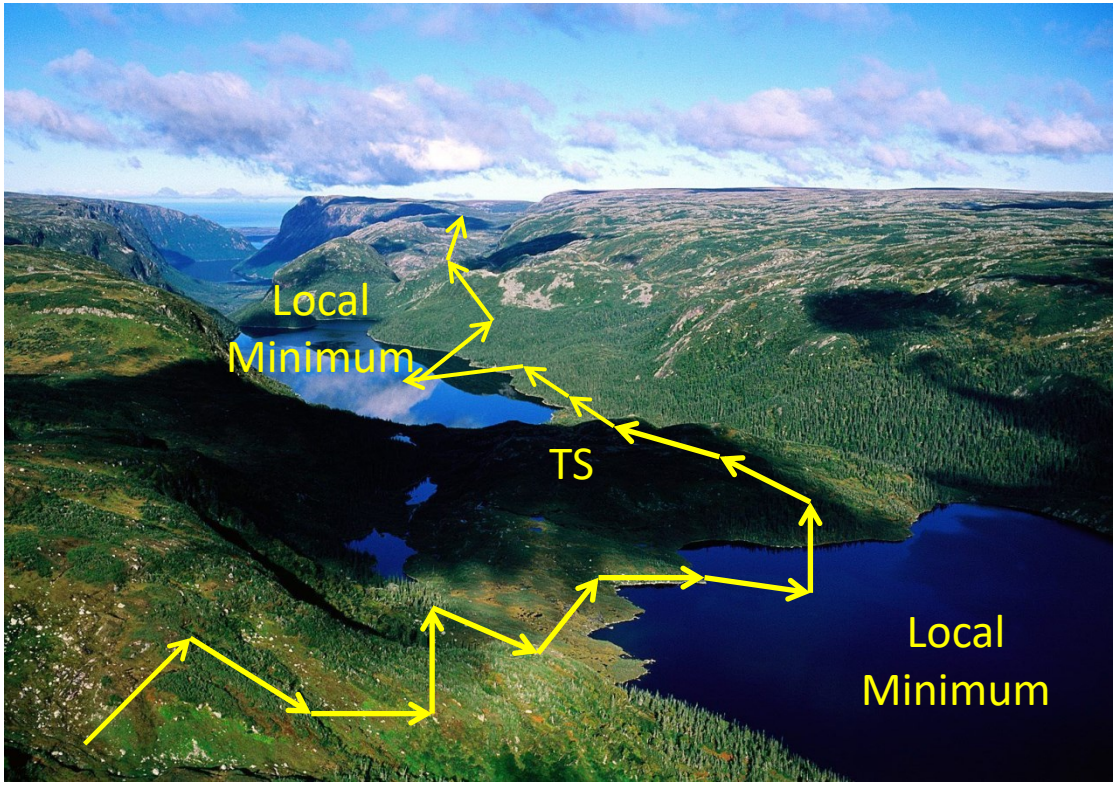
Surface aggregation and penetration destabilizes membrane, leading to poration

(Andersson, Ulmschneider, Ulmschneider, & White, 2013; Perrin & Pastor, 2016; Sengupta, Leontiadou, Mark, & Marrink, 2008; J. P. Ulmschneider, Smith, Ulmschneider, Ulrich, & Strandberg, 2012; M. B. Ulmschneider et al., 2014; Upadhyay, Wang, Zhao, & Ulmschneider, 2015; Wang et al., 2014)

PES Sampling by Basin-hopping



$$\tilde{E}(\mathbf{X}) = \min\{E(\mathbf{X})\}$$



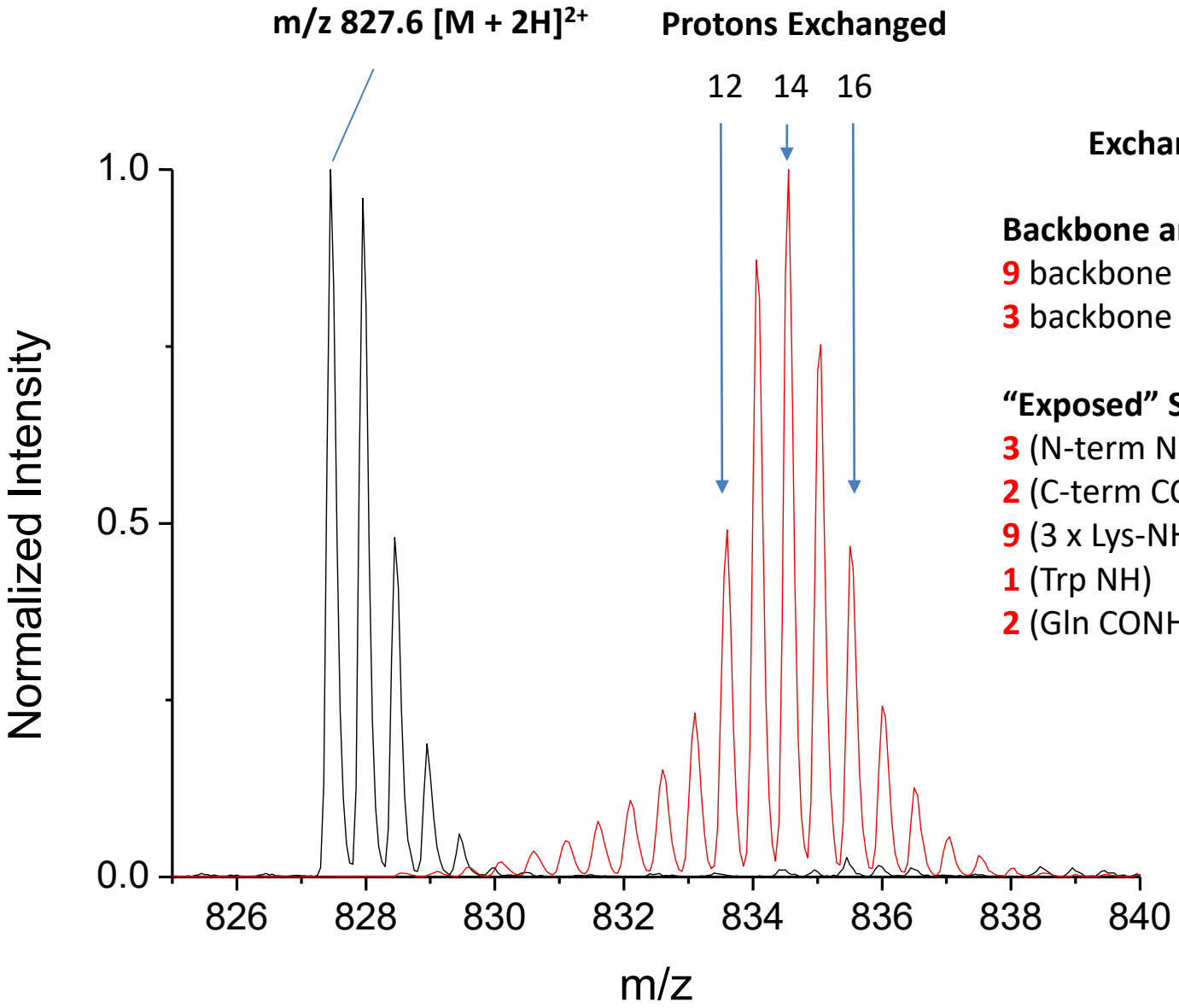
Ya gotta visit Gros Morne, Newfoundland b'y!

$\mathbf{X}$ represents a 3*N*-dimensional vector of nuclear coordinates

min signifies that an energy minimization is performed starting from $\mathbf{X}$

Criteria may be introduced to the basin hopping routine to limit PES regions sampled

DMS-HDX also Indicates Helical, Bridged MP1 Structure



Exchangeable Protons

Backbone amide:
9 backbone NH (helix)
3 backbone NH (coil)

12

"Exposed" Side Chain
3 (N-term NH_3)
2 (C-term $CONH_2$)
9 (3 x Lys-NH_3)
1 (Trp NH)
2 (Gln $CONH_2$)

17

Total: 29

Proton Abstraction Correlates with Gas-Phase Proton Affinities