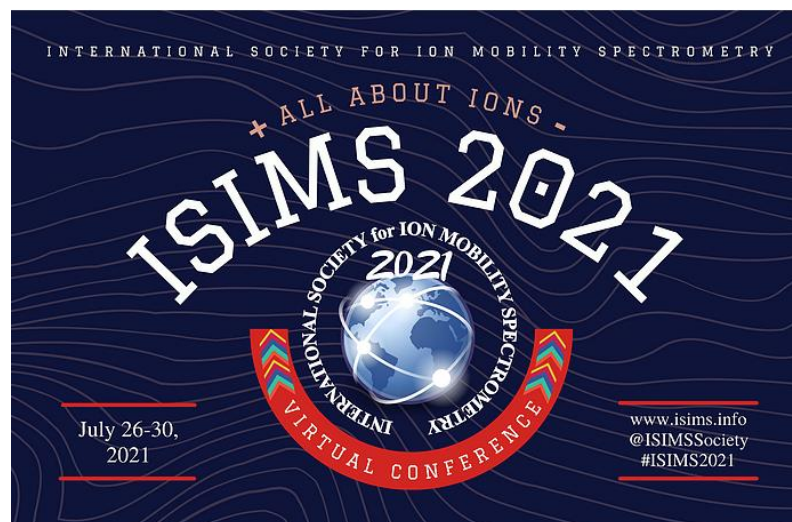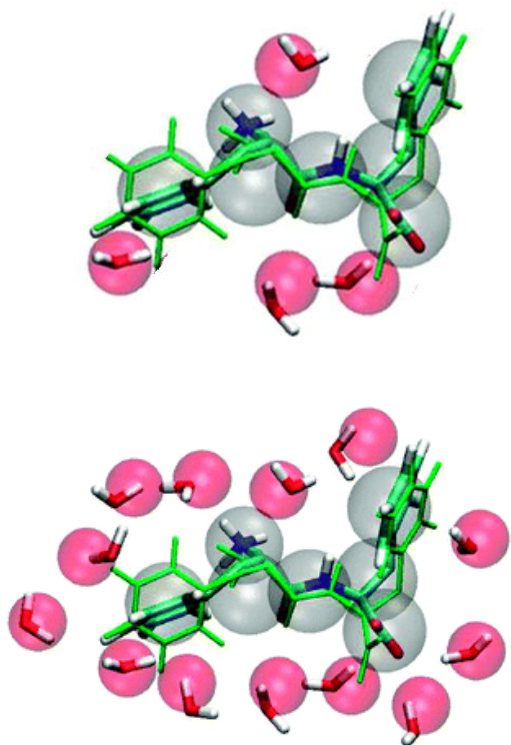# The Charge-State and Structural Stability of Peptides Conferred by Microsolvating Environments in DMS/FAIMS

Christian Ieritano

Hopkins Group
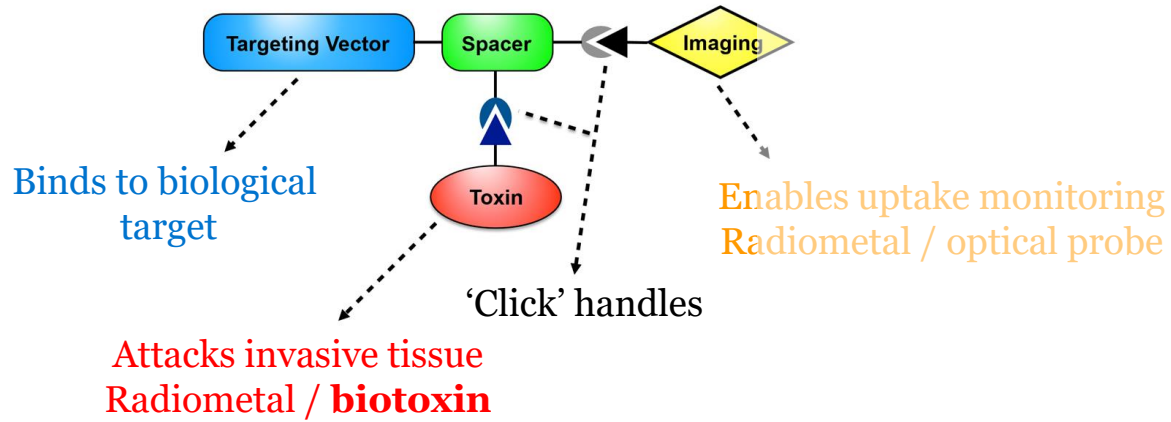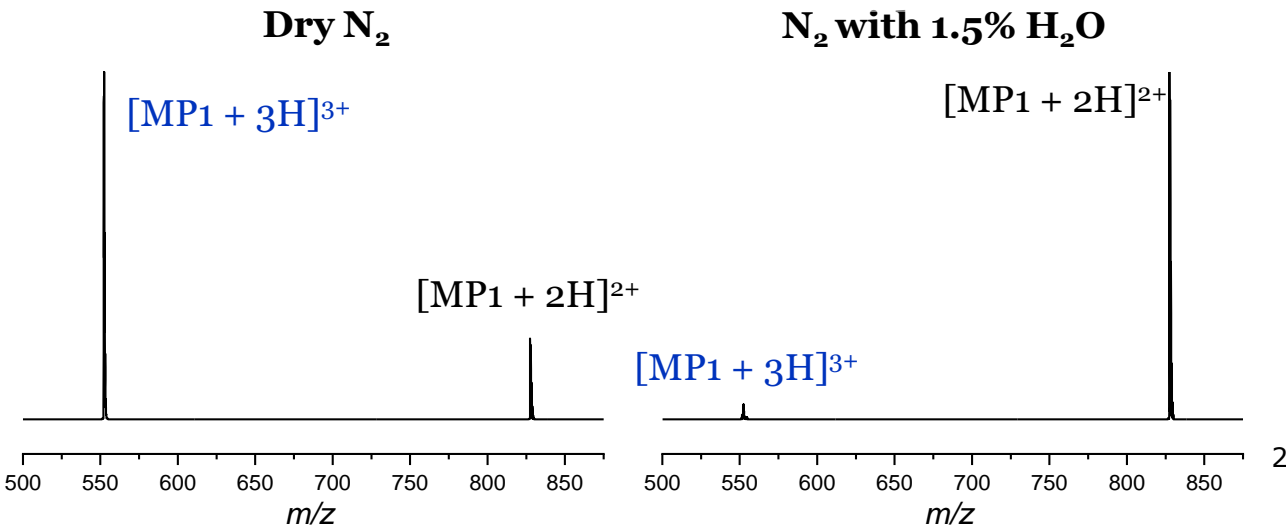
ISIMS 2021 Virtual Conference

JASMS, 2021, 32, 956-968

Back in my undergraduate days as a radiochemist…

Theranostic Agents (Therapeutic + Diagnostic)

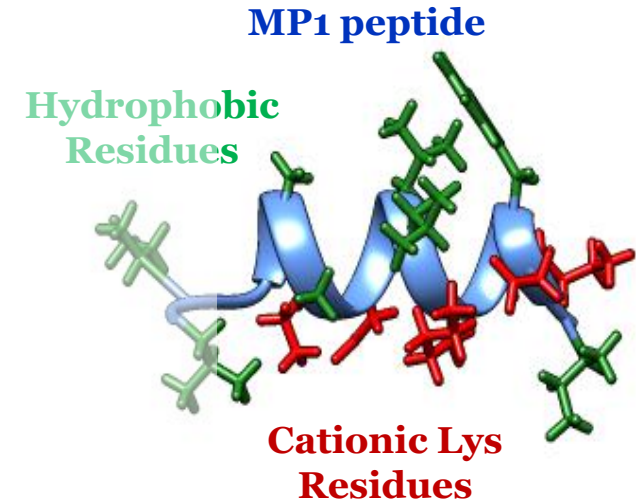**Binds to biological target**

Enables uptake monitoring
Radiometal / optical probe

'Click' handles

Attacks invasive tissue
Radiometal / biotoxin

Wasp-venom peptide (MP1) selectively targets cancer cells

MP1 peptide

Hydrophobic Residues

https://phys.org/news/2015-09-brazilian-wasp-venom-cancer-cells.html

Cationic Lys Residues

DMS-MS experiments gave some strange results

Dry N$_2$

[MP1 + 3H]$^{3+}$

[MP1 + 2H]$^{2+}$

500 550 600 650 700 750 800 850
m/z

N$_2$ with 1.5% H$_2$O

[MP1 + 2H]$^{2+}$

[MP1 + 3H]$^{3+}$
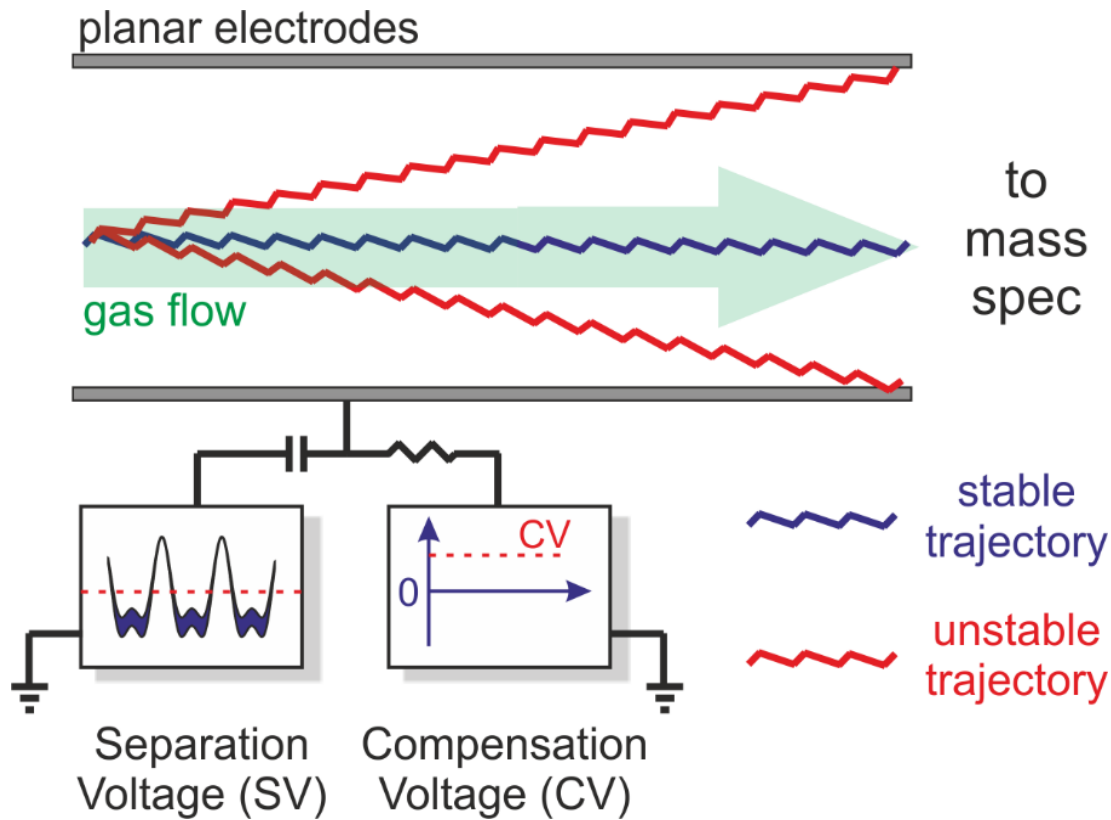
500 550 600 650 700 750 800 850
m/z

Solvent vapour alters the charge state distribution of MP1

Shift from +3 to +2 is mediated by microsolvation

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2

The DMS is a dynamic microsolvation environment

planar electrodes

to mass spec

gas flow

stable trajectory

unstable trajectory

CV

0

Separation Voltage (SV)　　Compensation Voltage (CV)

Doping the carrier gas with solvent vapour (*e.g.*, water) induces dynamic solvation/desolvation cycles
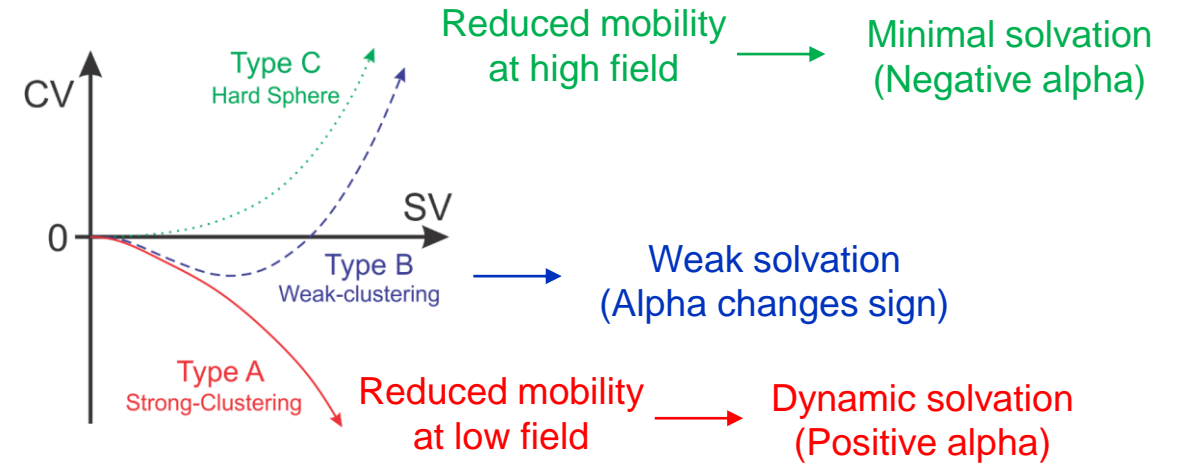
High field: "Hot" desolvated ions

Low field: "Cold," solvated ions
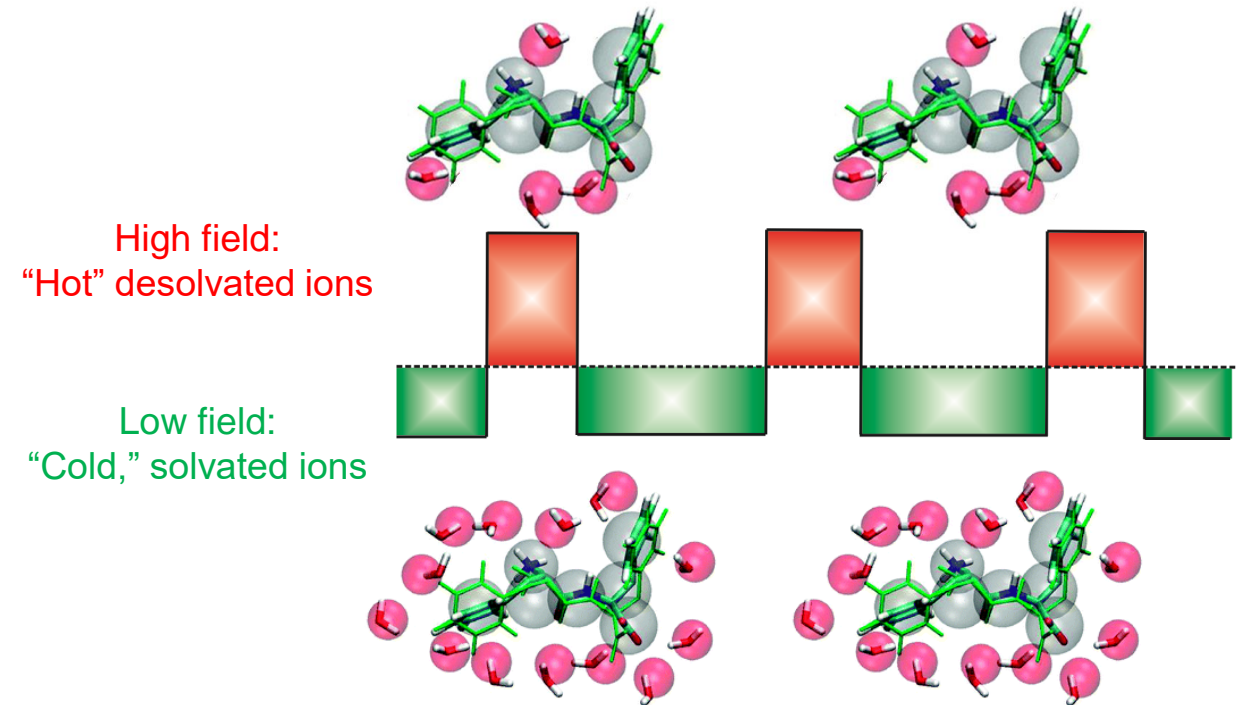
The magnitude and the sign of the **compensation voltage** (CV) is indicative of the strength of **ion-solvent interactions.**

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The DMS is a dynamic microsolvation environment

For a specific SV:

$$CV \propto \alpha(E) \qquad \alpha(E) = \frac{K(E)}{K(0)} - 1$$

DMS behaviour is a measure of the degree of microsolvation

CV

Type C
Hard Sphere

Reduced mobility at high field → Minimal solvation (Negative alpha)

SV

0

Type B
Weak-clustering → Weak solvation (Alpha changes sign)

Type A
Strong-Clustering

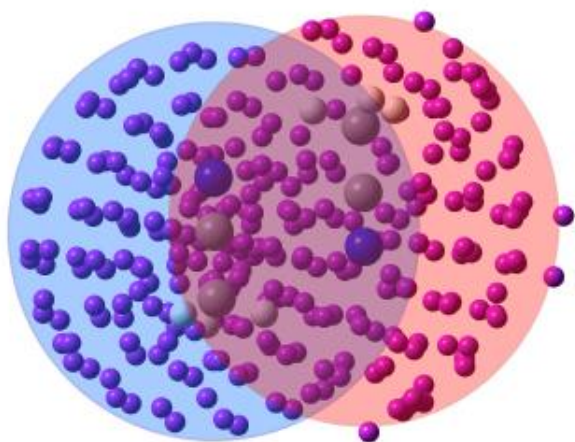Reduced mobility at low field → Dynamic solvation (Positive alpha)

Microsolvation occurs at charge sites. For MP1, these are the **3 Lys residues** and the **N-terminus**

We approximate these interactions as occurring on isolated Lys side chains $[\mathrm{PrNH_3}]^+$
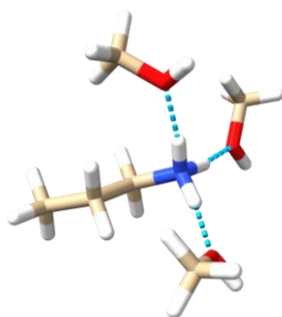

4
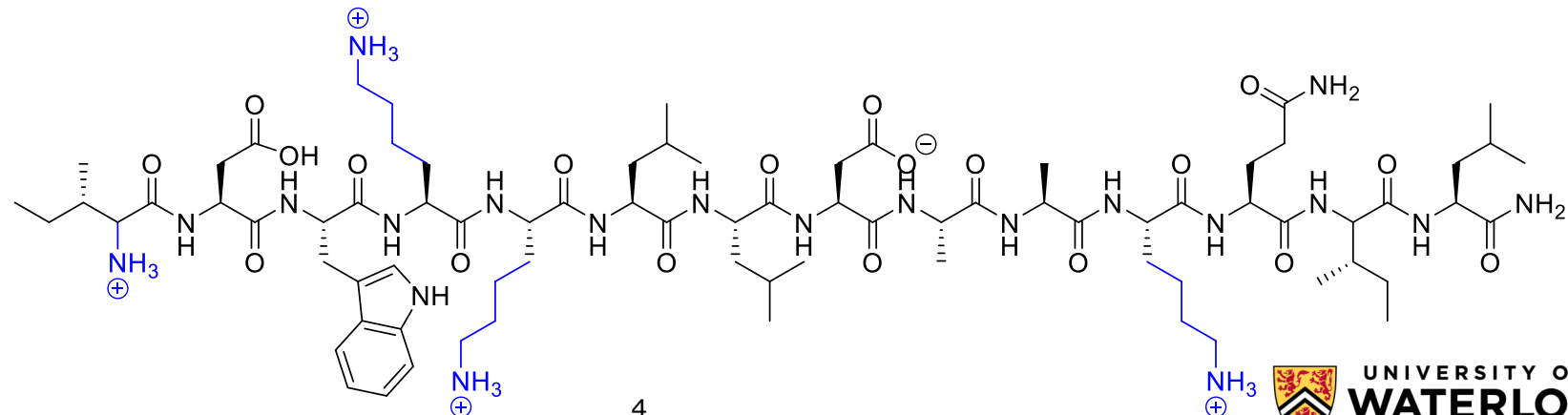
Modelling ion-solvent interactions within the DMS
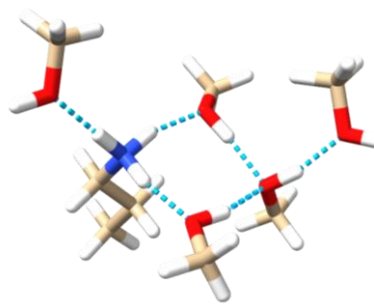
Systematic Sampling of Cluster Surfaces (SSCS)

$[PrNH_3\cdots n(MeOH)]^+$ clusters
Protic modifier model
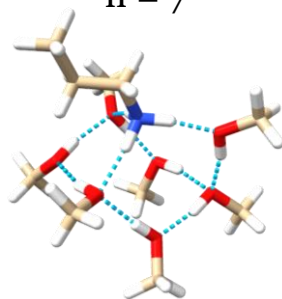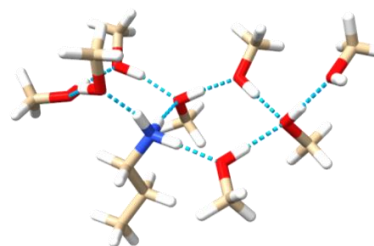
$[PrNH_3\cdots n(MeCN)]^+$ clusters
Aprotic modifier model

SSCS efficiently generates candidate geometries of microsolvated clusters

n = 3   n = 5

n = 7   n = 8
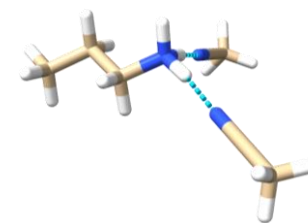
n = 2   n = 3
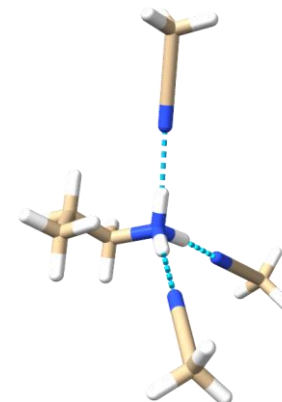
n = 5

Refinement by DFT [ωB97X-D3/6-311++G(d,p)] allows calculation of cluster thermochemistry (ΔG)

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Modelling ion-solvent interactions within the DMS

Multiple configurations for each cluster means that each geometry must be accounted for

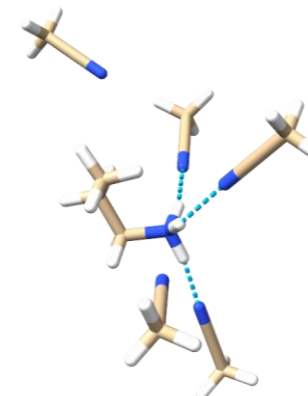$$\Delta G_{ass} = G_{[PrNH_3\cdots nSolv]^+} - \left(G_{[PrNH_3]^+} + nG_{solv}\right)$$
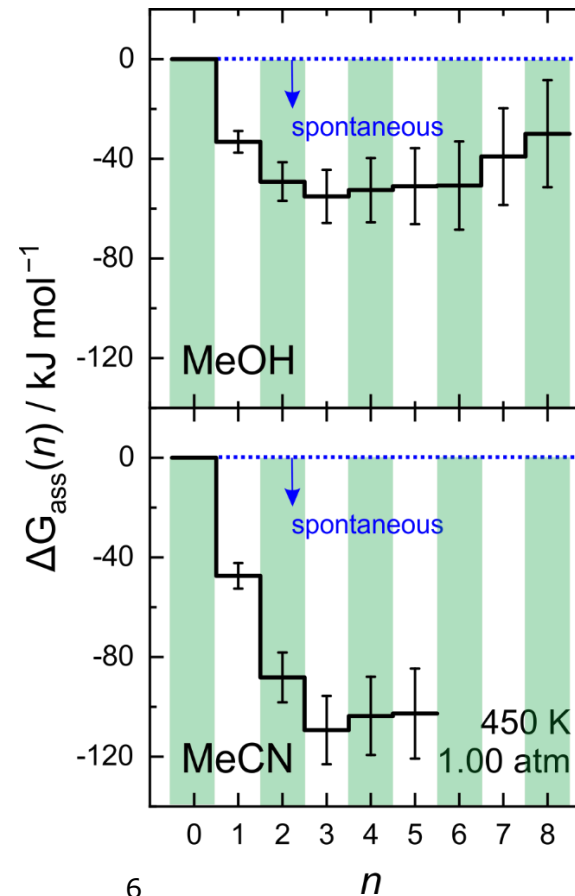
For a specific cluster of size n at temperature T, the total Gibbs energy is given by each isomer k

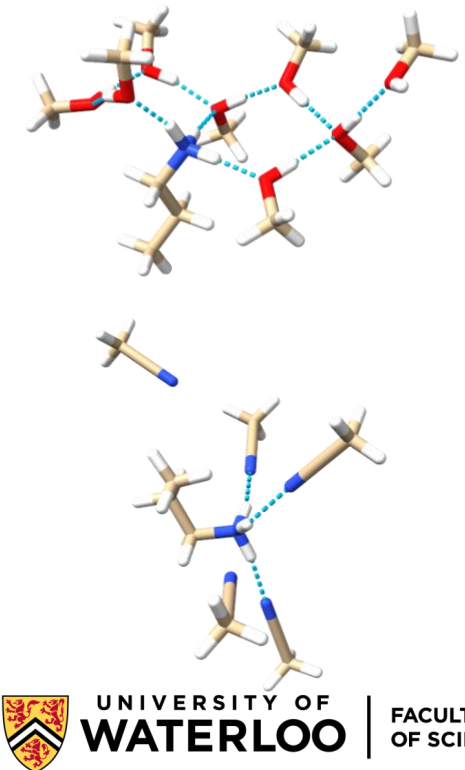$$G_n(T) = \sum_k \rho_n^{(k)}(T) \cdot G_n^{(k)}(T) \qquad G = H - TS$$

$$\rho_n^{(k)}(T) = \frac{\exp(-G_n^{(k)}(T) \cdot (k_b \cdot T)^{-1})}{\sum_k \exp(-G_n^{(k)}(T) \cdot (k_b \cdot T)^{-1})}$$

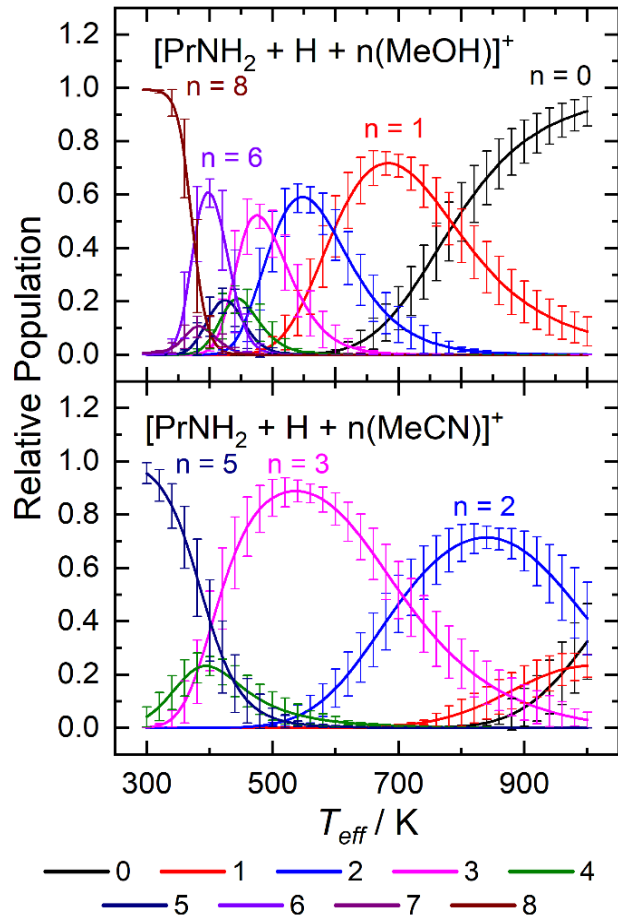$$\sum_k \rho_n^{(k)}(T) = 1$$

Protic vs. aprotic microsolvation occurs with different binding affinity

Modelling ion-solvent interactions within the DMS

Gibbs energies allow us to model the size of ion-solvent clusters as a function of the ion's temperature

$$N_i = N \cdot e^{-\Delta G_{ass_i}(T)/k_B T} \qquad N = \frac{1}{\sum_i N_i}$$

Microsolvation provides MP1 with a solvent 'air bag,' sheltering it from fragmentation

Cooling effect is two-fold:

Reduced mobility of ion-solvent cluster

Field-heating results in evaporation of solvent instead of fragmentation
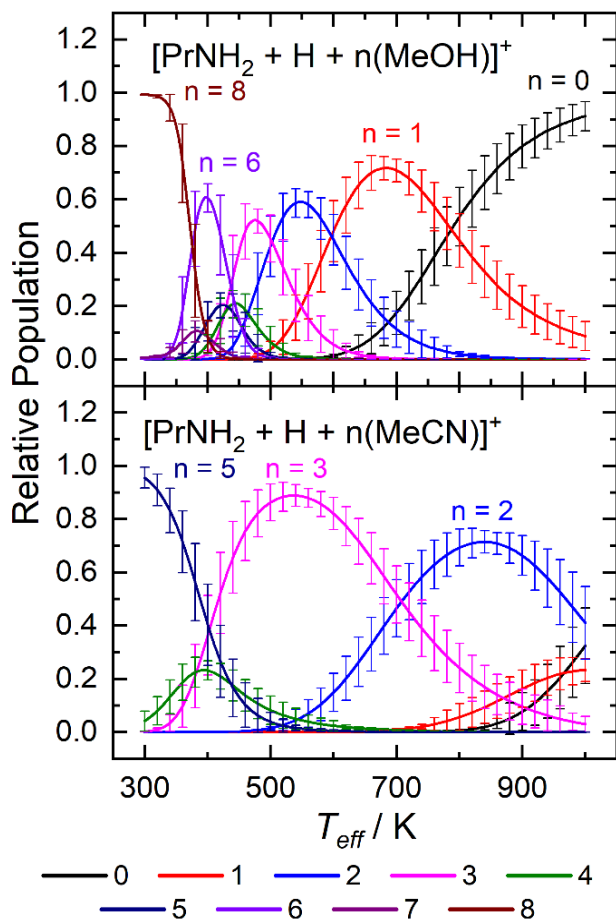
Can use ion-solvent cluster populations to calculate reduction in ion effective temperature (T_{eff})

$$T_{eff} = T_{bath} + T_{field} \approx T_{bath} + \frac{M}{3k_b}(KE)^2$$

7
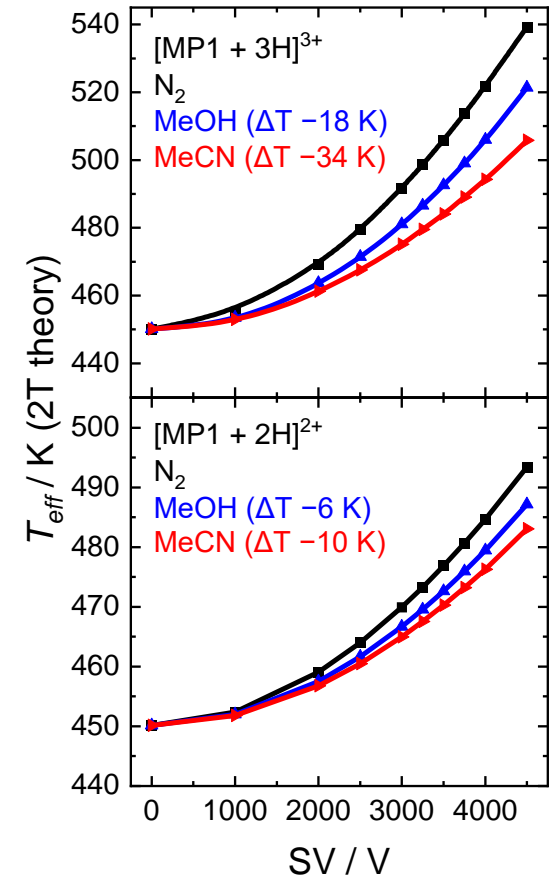
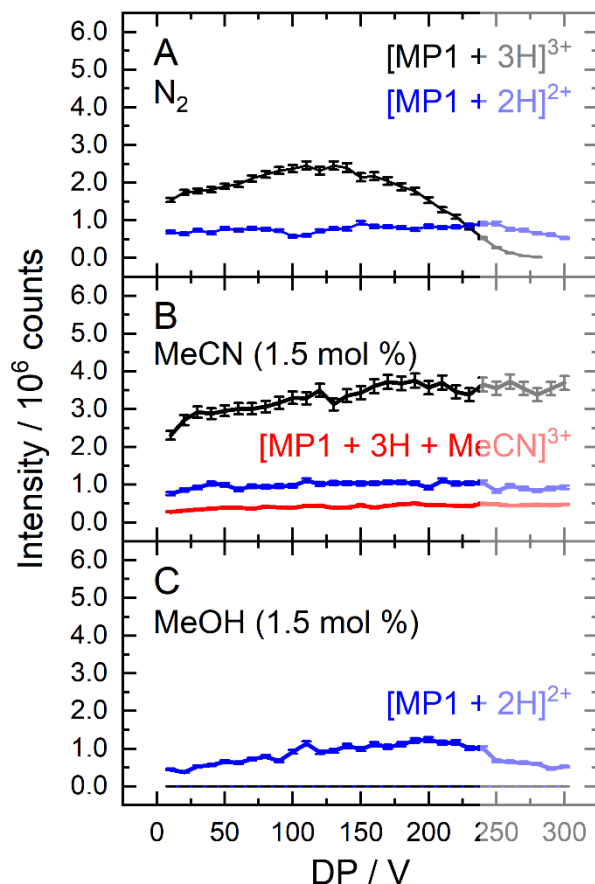# Microsolvation affords charge state and parent ion stabilization

Cluster populations

$[PrNH_2 + H + n(MeOH)]^+$

n = 8
n = 6
n = 1
n = 0

Relative Population

$[PrNH_2 + H + n(MeCN)]^+$

n = 5
n = 3
n = 2

T_{eff} / K

— 0   — 1   — 2   — 3   — 4
— 5   — 6   — 7   — 8

Ion intensity as DP is increased

A
N_2
$[MP1 + 3H]^{3+}$
$[MP1 + 2H]^{2+}$

B
MeCN (1.5 mol %)
$[MP1 + 3H + MeCN]^{3+}$

Intensity / 10^6 counts

C
MeOH (1.5 mol %)
$[MP1 + 2H]^{2+}$

DP / V

Decay of +3 state as DP increases

No fragmentation with aprotic modifier

Also detection of +4 state (not shown)

No detection of +3 state with MeOH?

Also absent in any protic modifier

Where does the +3 state go in MeOH?

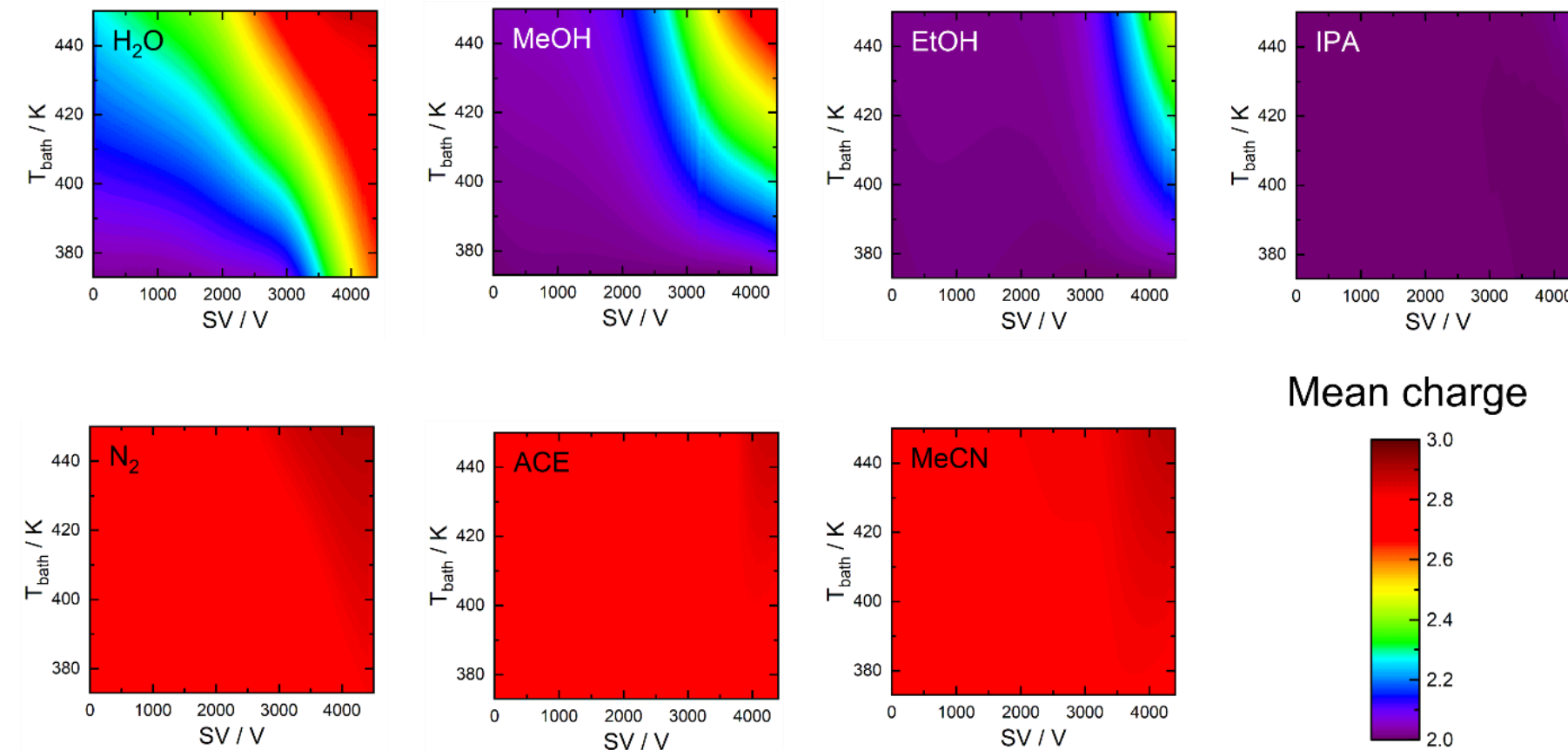$$B + H^+ \rightarrow BH^+ \qquad \Delta G = GPB$$
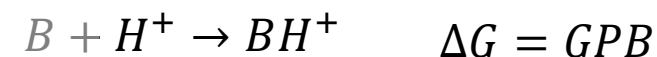
$$GPB_{eff}(T) = \sum_i N_i(T) \cdot GPB_i(T)$$

GPB_{eff} MeOH > MP1 ... but not always

GPB / kJ mol^{-1}

MeOH GPB_{eff}

T_{eff} / K

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8
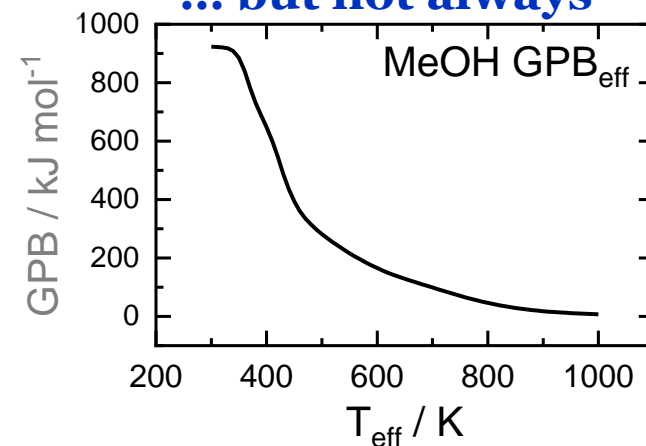
Microsolvation affords charge state and parent ion stabilization

Additional cooling by charge reduction at low SV

The +3 ion re-emerges at high bath gas temperatures and high SV fields
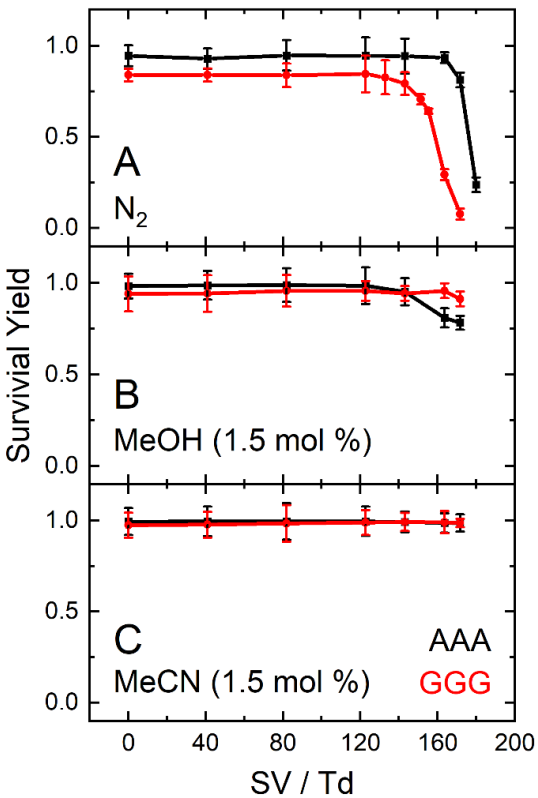
No charge transfer with aprotic modifiers. Why?
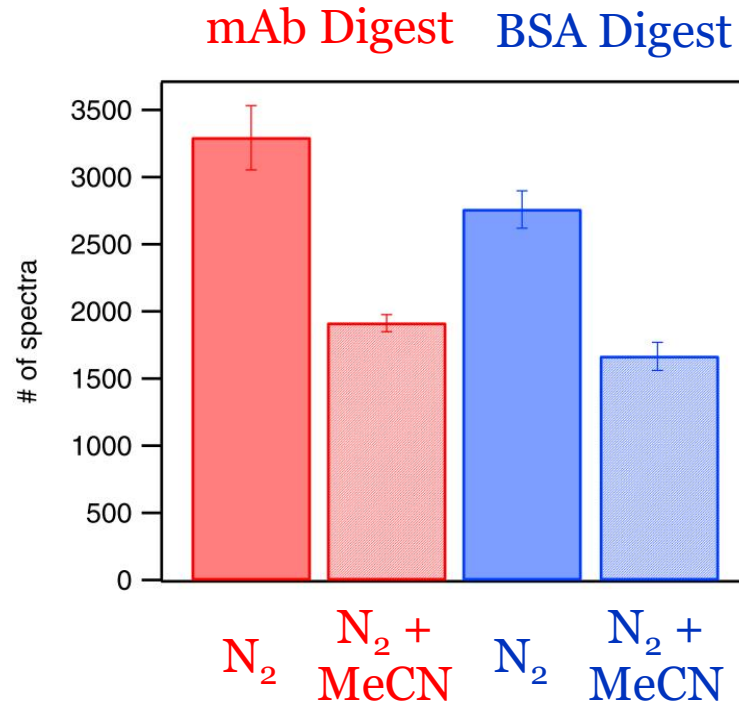
See Haack et al.
JASMS, 2020, 31, 785-795.

Gas-phase basicity ordering: **ACE** > IPA > **MeCN** > EtOH > MeOH > H_2O

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Peptide microsolvation has significant implications for DMS

Reduction in number of spectra required for DMS-based bottom-up proteomics

Anal. Bioanal. Chem. 2019, 411, 6365-6376

Survivial Yield

A — N$_2$

B — MeOH (1.5 mol %)

C — MeCN (1.5 mol %) — AAA — GGG

SV / Td

mAb Digest   BSA Digest

# of spectra

N$_2$ | N$_2$ + MeCN | N$_2$ | N$_2$ + MeCN

Preservation of native-like structures of biological ions in DMS can be mediated by microsolvation

ESI droplet

without microsolvation

with microsolvation

Non-native configuration

Side-chain collapse
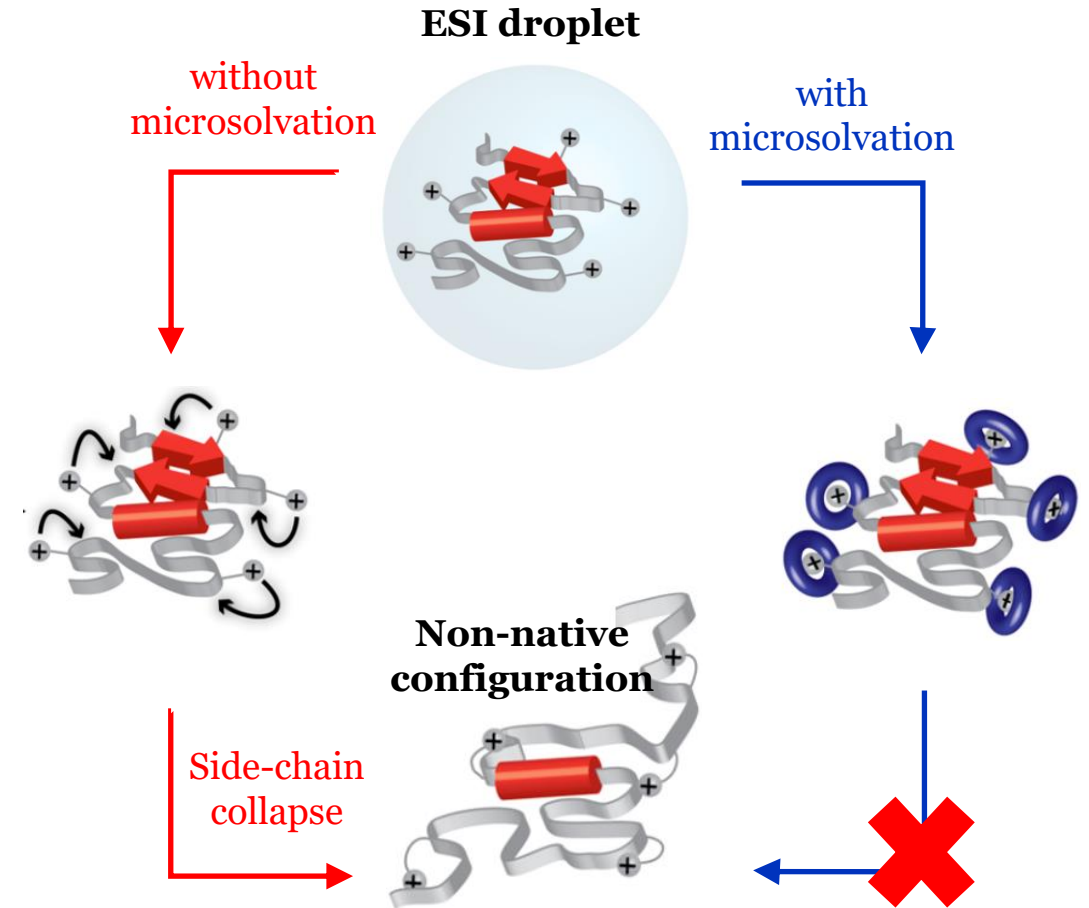
JACS. 2013, 135, 1177-1180

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Acknowledgements

PhD Committee

Prof. W. Scott Hopkins

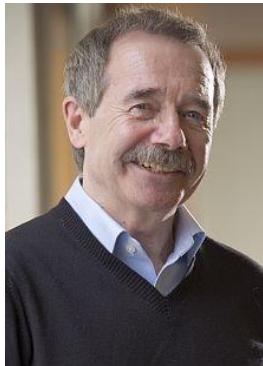Prof. Terry B. McMahon

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Dr. Brad Schneider
Dr. Mircea Guna

For more details, see our publication:

JASMS, 2021, 32, 956-968

Collaborators, Resources, and Funding

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