

Slice Collision-Induced Unfolding and Molecular Dynamics Reveal How Post-Translational Succination Reshapes SUMO1 Unfolding Pathways

Louis Groignet¹, Thomas Robert¹, Quentin Duez¹, Jehan Claessens², Patrick Brocorens¹, Pascal Gerbaux¹, Julien De Winter^{1*}

¹ University of Mons, UMONS, 20 Place du Parc, 7000 Mons, Belgium

² UCB, Braine-l'Alleud, Belgium

Overview

- **Purpose:** SUMO1 is a key modifier of proteins and may undergo succination via its reactive cysteine under conditions of metabolic stress. This study explores the structural implications of this PTM.
- **Methods:** ESI-(c)IMS/MS, CIU⁽ⁿ⁾, and Molecular Dynamics (MD).
- **Results:** Succination reshapes SUMO1 unfolding by stabilizing an intermediate, redirecting pathways *via* charge–dipole interactions.

Introduction

SUMOylation is a post-translational modification (PTM) in which Small Ubiquitin-like Modifier (**SUMO**) proteins are covalently attached to target proteins, regulating numerous cellular processes across more than a thousand substrates. [1,2] **Succination** is another PTM where **fumarate**, accumulating under metabolic stress or fumarase deficiency, irreversibly modifies **cysteine** residues to form 2-(succino)cysteine (**2SC**) (**Figure 1**). [3] SUMO1 contains a reactive cysteine and can therefore be succinated, potentially impairing its SUMOylation function and contributing to disease. While SUMO1 diethyl (DE) succination has been shown to induce structural changes in solution, these effects are lost in the gas phase (CCS) due to compaction. [4]

Methods

cIMS-MS experiments were performed using a Waters Select Series cyclic. SUMO1/2SC-SUMO1 solutions (5 μ M, ~11 kDa) in 50 mM ammonium acetate were analyzed by nanoESI+. $^{TM}CCS_{N_2 \rightarrow He}$ values were obtained using polymer calibration on a Waters Synapt G2-Si. [5] CIU¹ uses a trap CV ramp to unfold selected ions, while CIU² applies a second activation step after IMS-based conformer selection in the cIMS. **MD simulations** were performed on SUMO1 (PDB 1A5R) with AMBER (ff19SB/GAFF2/OPC), including 500 ns in solution followed by 500 ns in the gas phase, repeated with thermostat temperature ranging from 300 to 900 K. The final 500 MD conformations were analyzed with Collidoscope (Trajectory Method) to calculate theoretical $^{TM}CCS_{He}$. [6]

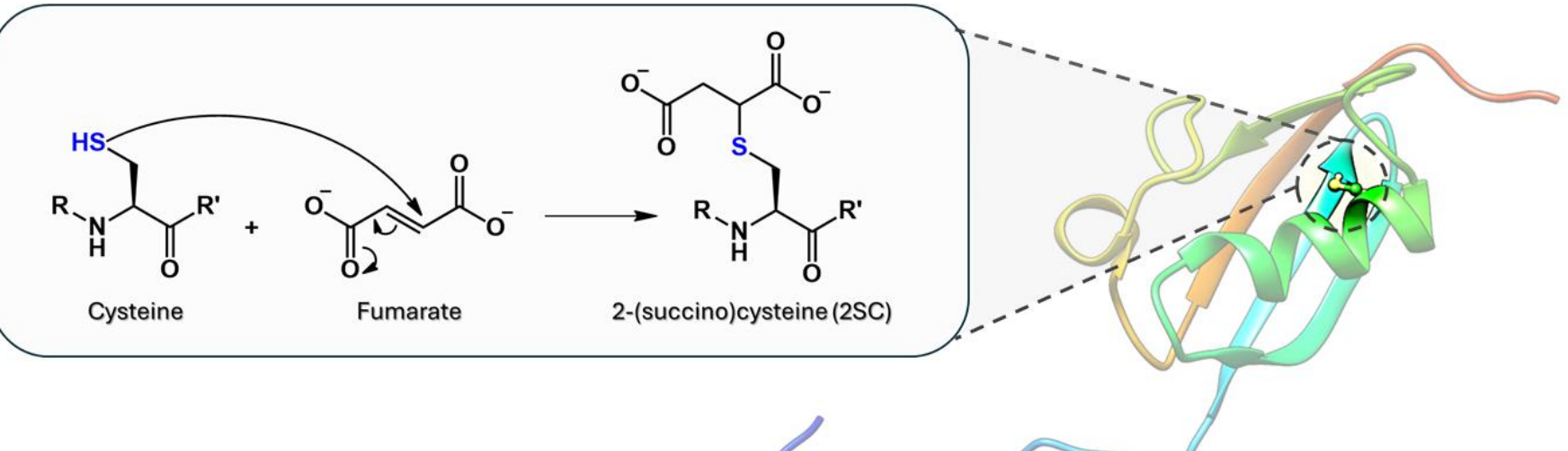


Figure 1: Succination reaction between SUMO1 (PDB 1A5R) cysteine, located in its α -helix, and fumarate forming 2-(succino)cysteine SUMO1 (2SC-SUMO1).

Results: Experimental section

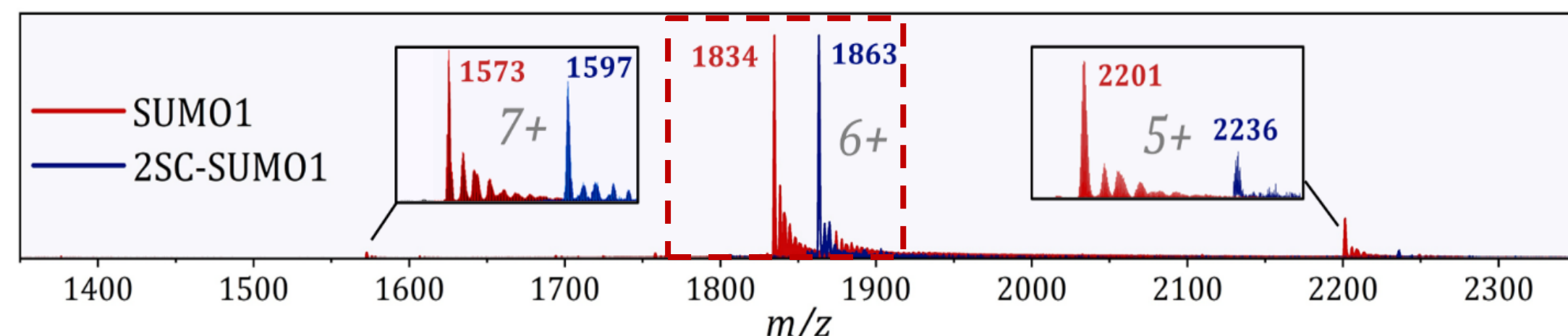


Figure 3: ESI(+) MS of the SUMO1 / DEF in 50 mM AA after 24 hours at 37 °C. **Red labels** correspond to SUMO1 and **blue ones** to 2SC-SUMO1. This spectrum demonstrates the spontaneous in vitro DE succination.

CIU¹ analysis

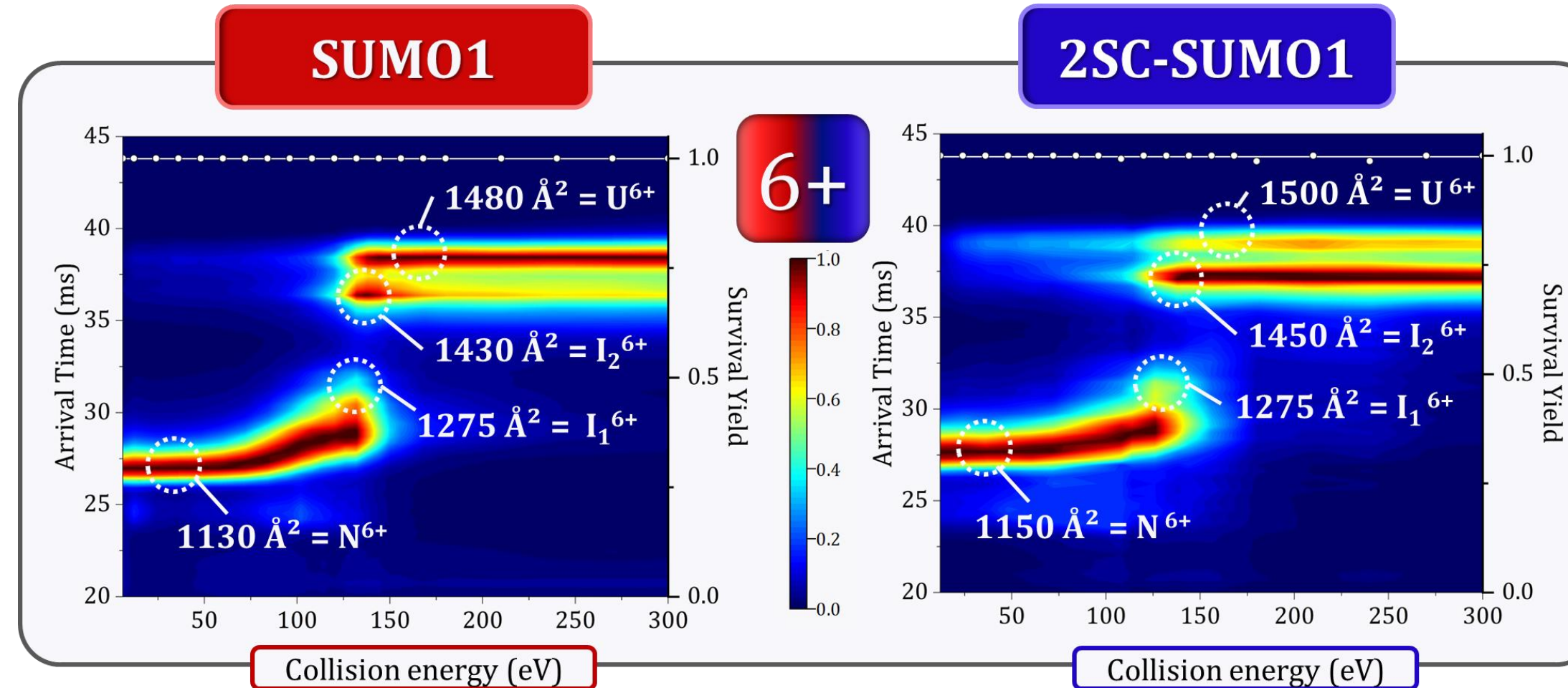


Figure 4: CIU experiments on the of SUMO1/2SC-SUMO1 6+ ions. Fingerprints are presented as a zoom on the unfolding region (0 to 50 V). N, I and U stand for Native, Intermediate and Unfolded.

CIU² analysis

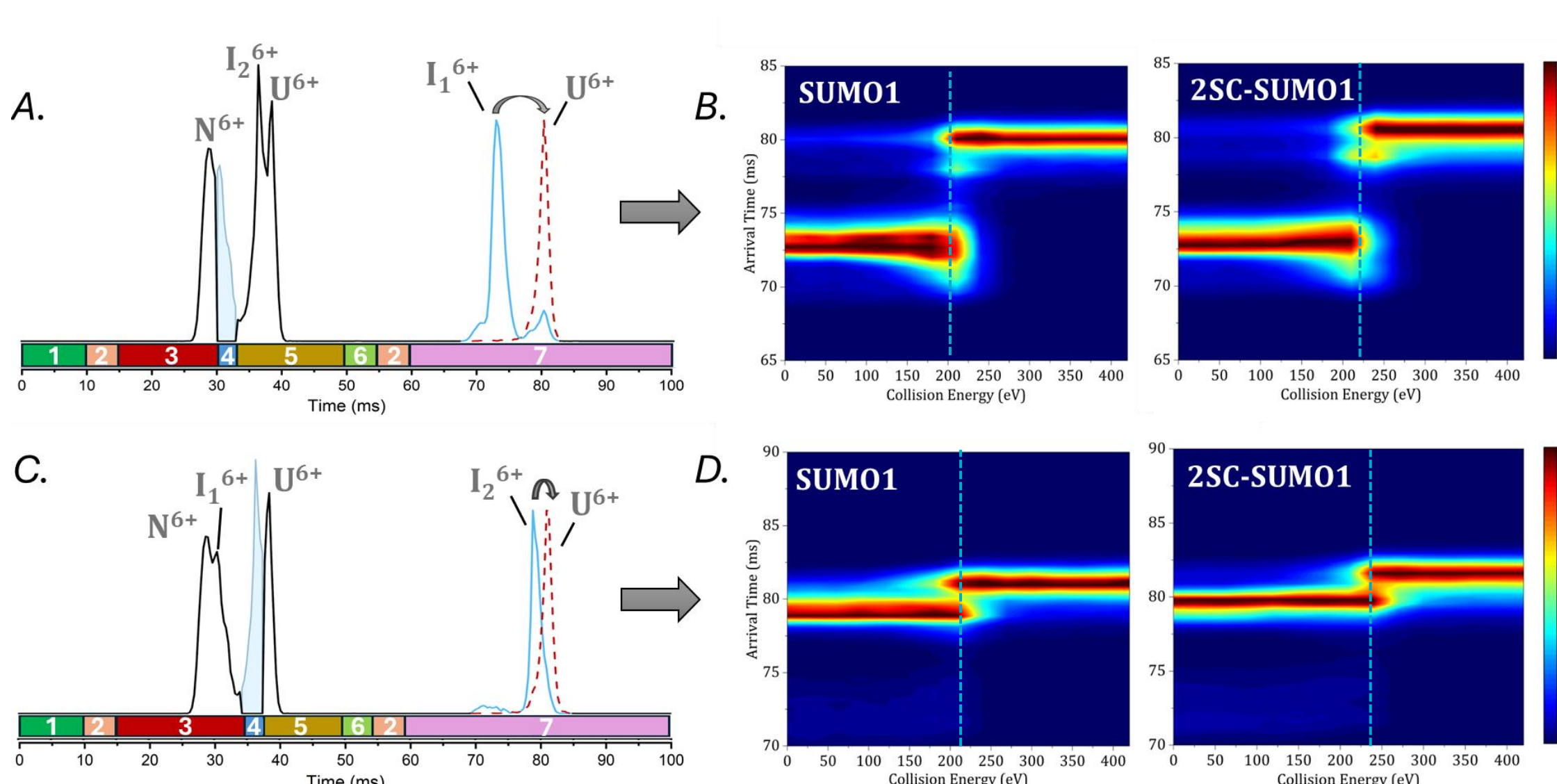


Figure 5: Schematic of the cIMS sequence in CIU² experiments for I₁⁶⁺ (A) and I₂⁶⁺ (C): 1 Inject, 2 Separate, 3 Eject, 4 Eject to prestore (blue conformation), 5 Hold and eject, 6 Reinject from prestore (RV yields U⁶⁺, red), 7 Eject and acquire. (B) CIU² fingerprints of the I₁⁶⁺ (31–33 ms) conformer of SUMO1 and 2SC-SUMO1. (D) CIU² fingerprints of the I₂⁶⁺ (34–37 ms) conformer of SUMO1 and 2SC-SUMO1.

Experimental setup (Waters Select Series cyclic)

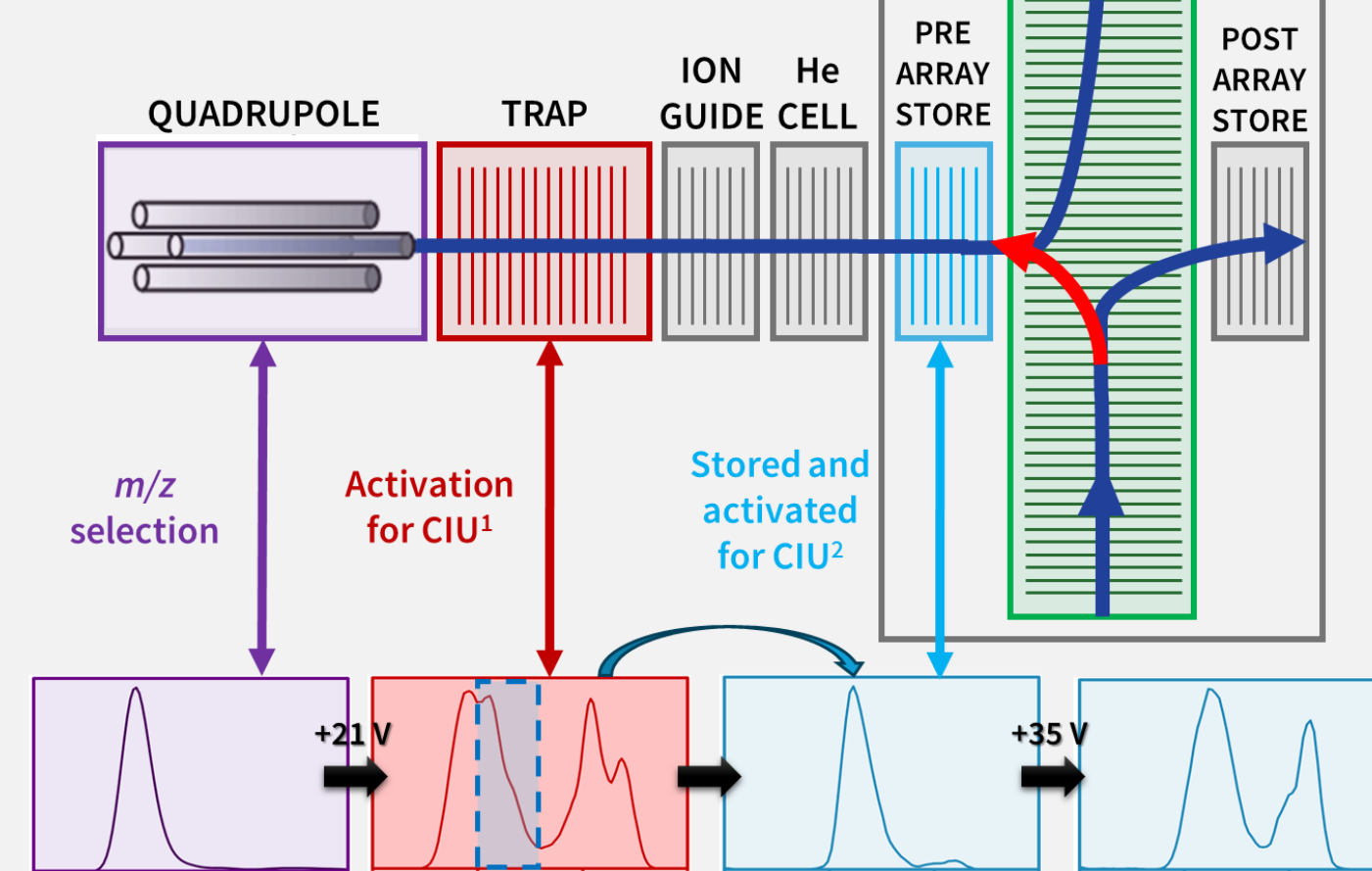


Figure 2: Cyclic IMS apparatus for CIU². Figure adapted from ref [7].

Stability parameters (CIU₅₀ and CE₅₀) for the 6+ charge state of SUMO1 and 2SC-SUMO1.

	CIU ₅₀ (eV)	CID ₅₀ (eV)
SUMO1	93.4 ± 1.5 & 136.6 ± 0.2	621.8 ± 1.0
2SC-SUMO1	93.2 ± 2.7 & 131.4 ± 1.5	625.2 ± 0.6

CIU² Analysis of the 6+ ions reveals unfolding mechanisms

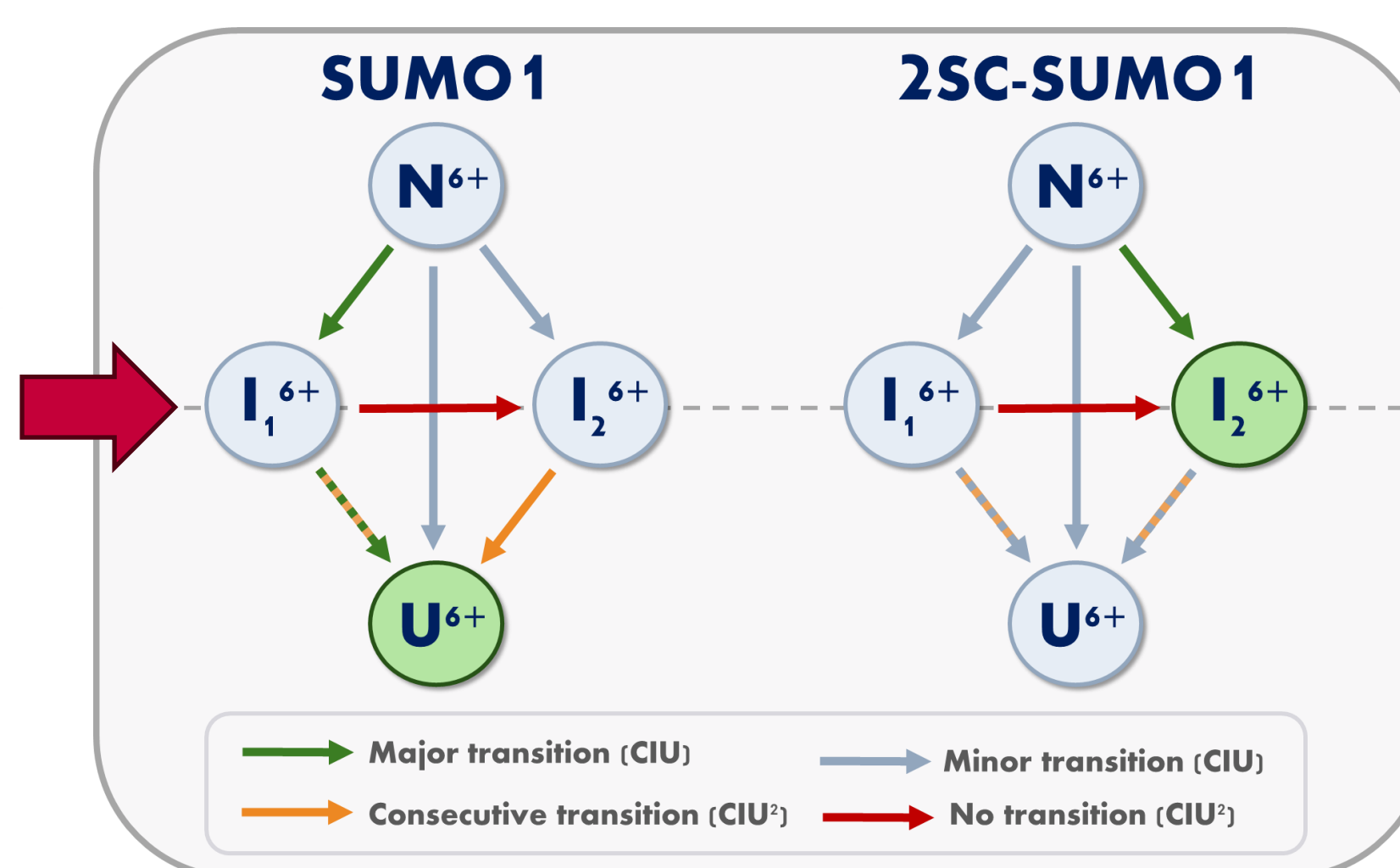


Figure 6: Mechanistic proposal of the unfolding transitions observed in CIU¹ and CIU² for the SUMO1 and 2SC-SUMO1 6+ ions.

Results: Theoretical section

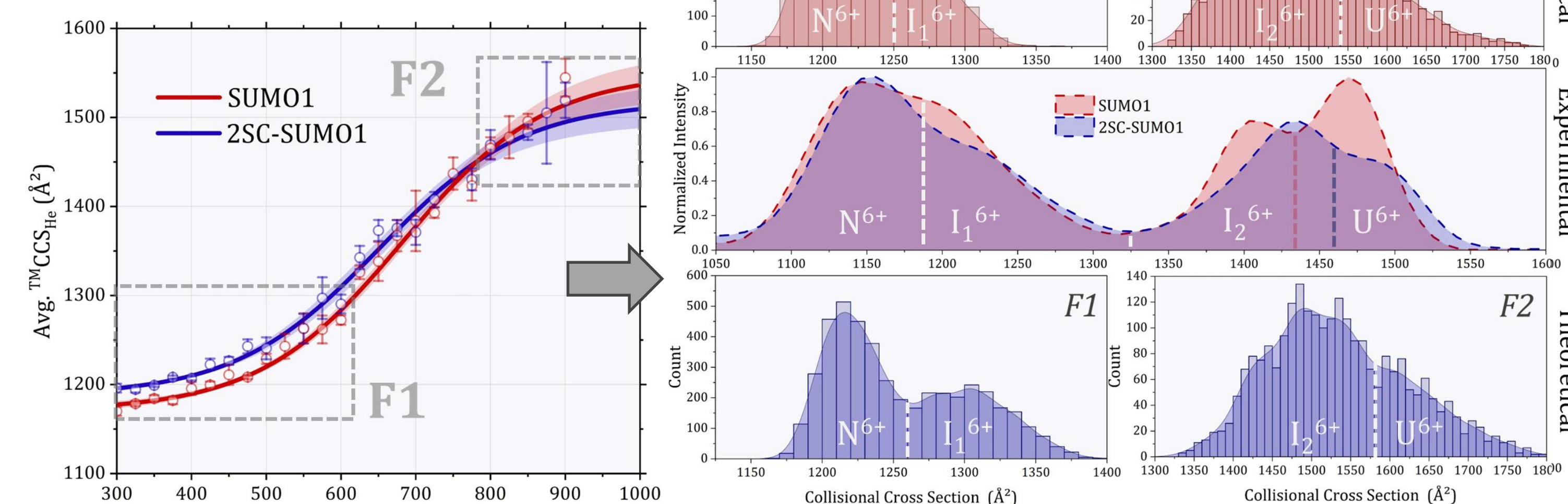


Figure 7: Boltzmann sigmoid fit of averaged $^{TM}CCS_{He}$ evolution (300–900 K, 25 K steps) from the final 250 ns of in-vacuo MD simulations (n = 3) for SUMO1 and 2SC-SUMO1 (6+ charge state, R² = 0.99). F1 and F2 denote the two main features.

Figure 8: Top panels show smoothed $^{TM}CCS_{He}$ histograms (10 Å² bins) for SUMO1 (red) for F1 and F2, while bottom panels display the corresponding distributions for 2SC-SUMO1 (blue). The middle panel presents experimental $^{TM}CCS_{N_2 \rightarrow He}$ distributions at CIU₅₀. Conformational states N⁶⁺, I₁⁶⁺, I₂⁶⁺, and U⁶⁺ are assigned to the different regions.

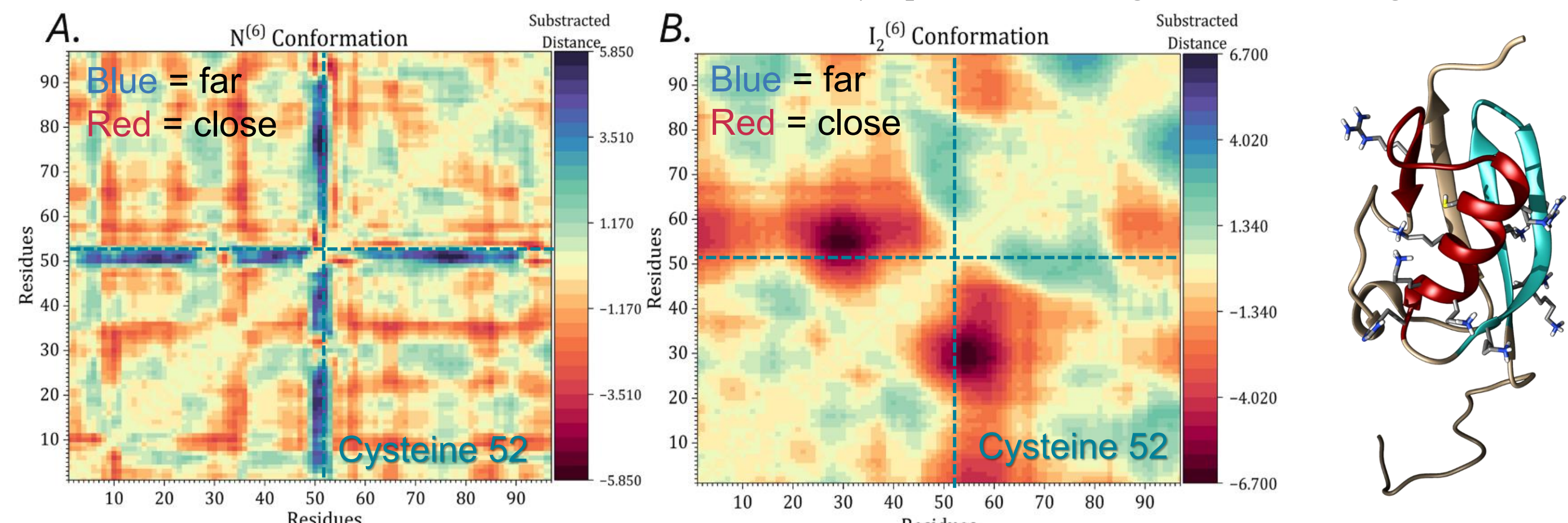


Figure 9: Inter-residue distance difference heatmap of the N⁶⁺ conformation (A) and the I₂⁶⁺ conformation (B). These heatmaps are the result of the subtraction of the inter-residue distance of 2SC-SUMO1 – SUMO1. Blue represents longer distance (opening) due to DE succination, while red represents shorter distance (compaction). DE succinated cysteine at residue 52.

Conclusions

DE succination reshapes the gas-phase unfolding landscape of SUMO1 by altering unfolding pathways and stabilizing specific intermediate states, despite minimal changes in global stability indicators. CIU² and MD reveal that this effect arises from selective stabilization of a compact intermediate *via* non-native charge–dipole interactions, supporting a kinetic-routing model where PTMs bias conformational pathways rather than overall stability.

References

- [1] Melchior *et al.* (2007) Nat. Rev. Mol. Cell Biol., 8 (12), 947–956
- [2] Goldberg *et al.* (2019) J. Biol. Chem., 294 (42), 15218–15234
- [3] Frezza *et al.* (2024) Trends Biochem Sci., 49 (9), 775–790
- [4] Groignet *et al.* (2025) ACS Pharmacol. Transl. Sci., 8 (8), 2683–2693
- [5] Duez *et al.* (2017) J. Am. Soc. Mass Spectrom., 28 (11), 2483–2491.
- [6] Prell *et al.* (2017) J. Am. Soc. Mass Spectrom., 28 (4), 587–596.
- [7] Makey *et al.* (2024) Anal. Chem., 96 (15), 6021–6029.
- [8] Eldrid *et al.* (2021) J. Am. Soc. Mass Spectrom., 32, 1545–1552.

Acknowledgments

The S²MOs and CMN labs are grateful to the FNRS for continuing support. Q. D. is a FNRS research fellow. T.R. thanks the FRIA for his Ph.D. grant. L.G. thanks the UMONS for his Ph.D. grant. The authors thank UCB for providing access to high-resolution cyclic IMS. J.C. is an employee of UCB and may hold company shares.