May post-translational succination be involved in cardiac arrhythmia?

A joint experimental and theoretical study combining CIU and molecular dynamics approaches

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Introduction:

In the Krebs cycle, the conversion of fumarate into malate by the addition of a water molecule is catalyzed by fumarate hydratase. In some diseases, there is a germline mutation of the fumarase gene resulting in an accumulation of fumarate due to an enzymatic deficiency [1]. This molecule can undergo a Michael addition with proteins bearing free thiol functions (free cysteines). This physiologically irreversible reaction is called succination (**Figure 1**). The products, called 2-succinocysteine residues are known to be involved in some pathologies such as diabetes or cardiomyopathies when they are overexpressed [2].

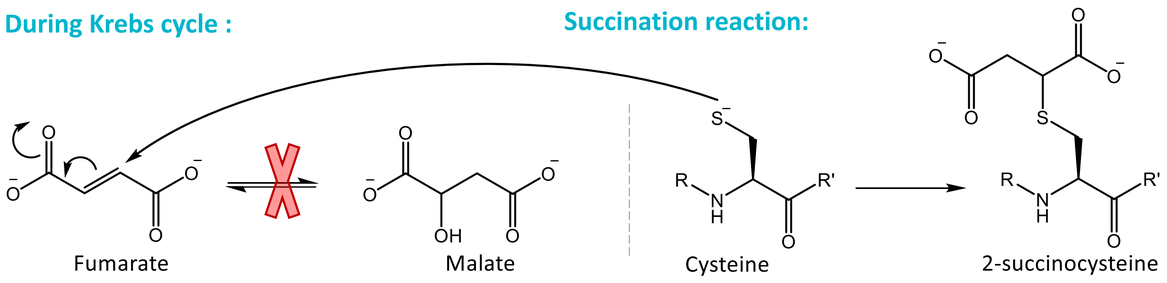

Figure 1: On the left, the deficiency of the hydration reaction catalyzed by the fumarate hydratase leads to an accumulation of fumarate in the cells. On the right the succination reaction between cysteine and the fumarate.

In this study, a Mass Spectrometry (MS)-based approach was selected as a potential alternative to current assays to highlight 2-succinocysteine. One of the targeted proteins of this study is SUMO1 (Small Ubiquitin-like MOdifier 1). This protein plays a key role in the stabilization of some proteins, including SERCA2a, that is involved in Ca^{2+} regulation during cardiac contraction (**Figure 2**) [3]. Thus, our main objective is to evaluate by Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS) (*via* Collision Induced Unfolding *'CIU'* and Collisional Cross Section *'CCS'* measurement) and molecular dynamics whether the succination of SUMO1 has an impact on its 3D structure/stability and therefore, whether it has an impact on its function (SUMOylation of SERCA2a) and induces cardiac arrhythmia.

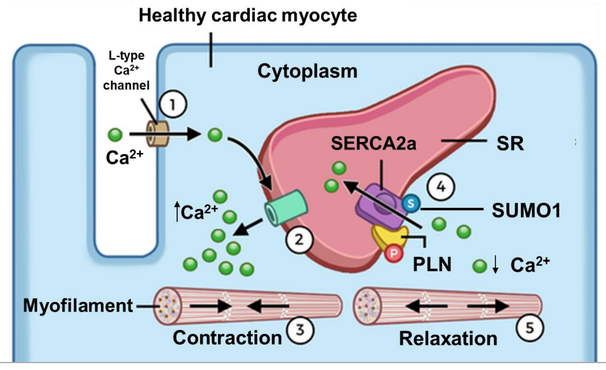

Figure 2: Scheme of SERCA2a function in the sarcoplasmic reticulum (SR) of cardiac myocytes. Upon a signal from a neurotransmitter, the SR releases Ca^{2+} ions into the cytoplasm, causing the heart to contract. These ions are then taken back by SERCA2a, a calcium-transporting ATPase, which allows the heart to relax. Figure adapted from Labster Theory website.

What is Collision Induced Unfolding (CIU) ?

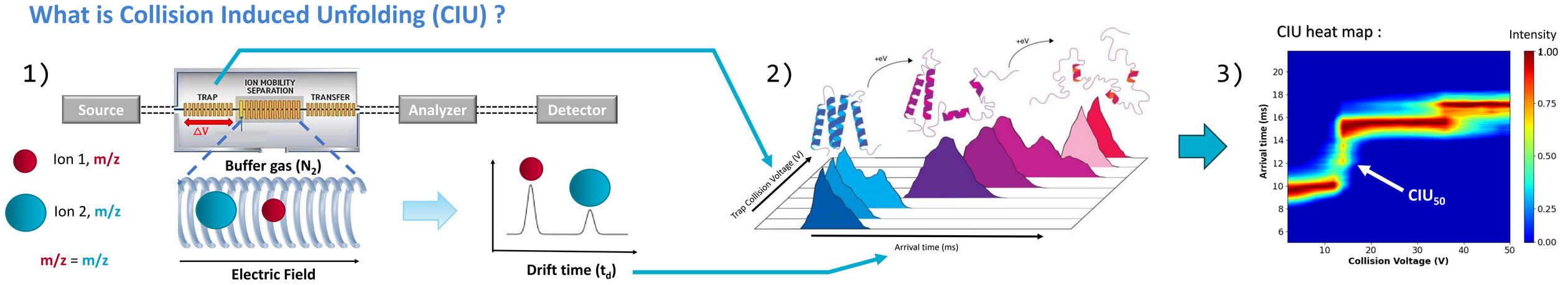

Figure 3: **1)** IMS-MS (Synapt G2-Si) apparatus is used to separate selected ions with the same *m/z* in function of their arrival time, characteristic of the 3D structure (typically a folded protein and an unfolded one). **2)** Then we can rise the trap collision voltage to increase the internal energy of ions through collisions with argon and then follow the unfolding of proteins thanks to the drift time. Figure coming from ref [4]. **3)** On this figure a typical heatmap of CIU is represented (myoglobin in this case) where the arrival time is plotted according to the trap CV using CIU*Suite*2 software [5].

What about SUMO1 ?

Experimental data

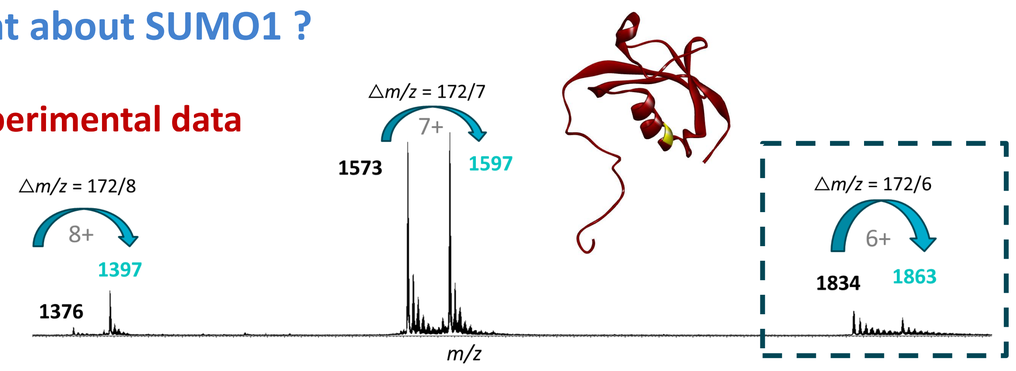

Figure 4: Native mass spectrometry of SUMO1 on SYNAPT G2-*Si* (positive mode). SUMO1 was incubated at 37°C (pH 7) for 24 hours with 100 molar equivalents of diethyl fumarate. The black-labeled signals correspond to the **SUMO1 protein**, and the blue-labeled ones represent the **succinated SUMO1**. The mass difference measured between these signals corresponds to the mass of DEF divided by the charge.

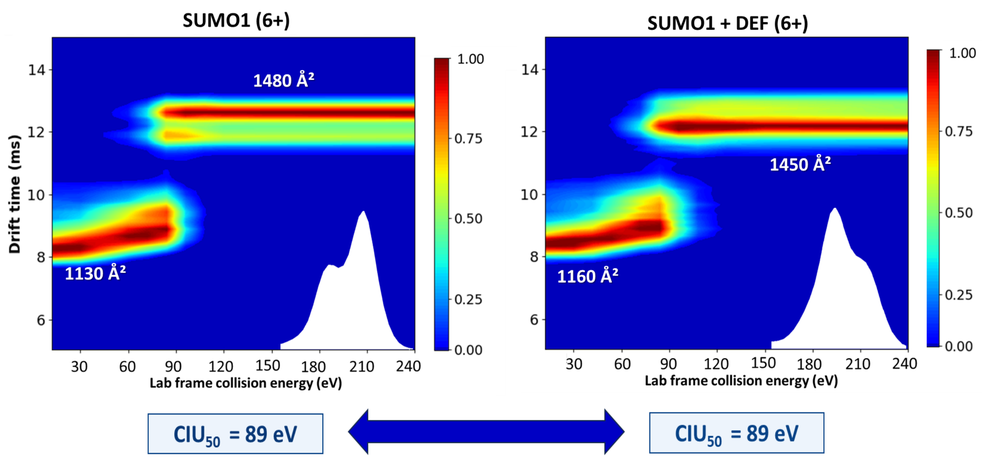

Figure 5: CIU heat map of SUMO1. On the left, before succination and on the right, after succination. At lower energies, the behavior of the two protein ions are the same, and the CIU_{50} (collision voltage necessary to convert 50 percent of a compact protein form into an energetically adjacent extended state) stay unaltered showing us that the succination doesn't really impact its 3D structure. This conclusion is also represented in CCS value, where a slight difference of 30 Å² is present.

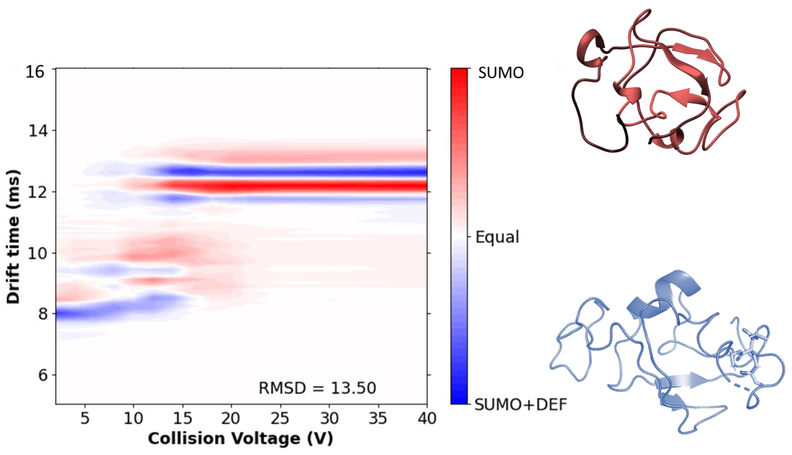

Figure 6: On this figure, the comparison between the CIU behavior of SUMO1 before and after succination is represented. The more the signal is red, the more the conformation corresponds to pristine SUMO1 ions, and blue to succinated SUMO1 ions. Even though suc. SUMO1 is shifted at higher ATD due to succination, what is interesting is the difference in population density. This difference is also visible on Fig 5. This suggests that succination change stability of SUMO1 at higher energies.

Theoretical data

To get structural information, the theoretical methodology was optimized to simulate as correctly as possible the protein structure in Ion Mobility experiments to determine theoretical CCS to compare to experimental results. The SUMO1 structure was uploaded from the protein data bank (pdb). Molecular dynamics (MD) simulations were performed to get structural information at the atomic level (1 µs in explicit water solvent + 2 µs in vacuum) (**Figure 7**). Simulations were carried out with the AMBER suite of programs, using the ff19SB force-field for the protein, GAFF2 for the DEF moiety and the OPC model for water. Conformations are extracted from MD and injected into the Collidoscope program to compute theoretical CCS (CCS_{th}) through the Trajectory Method (TM) [6]. This method is currently the most accurate to compute CCS and compare them to the CCS_{exp}.

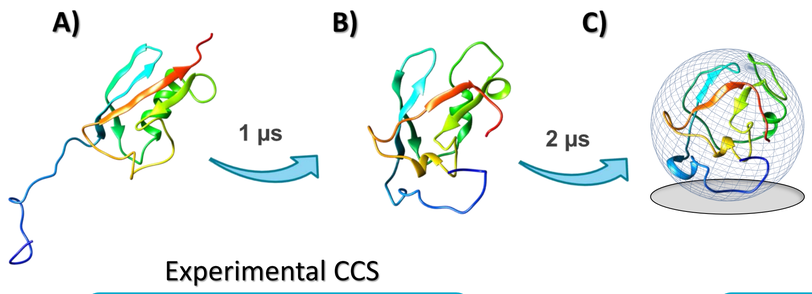

Figure 7: UCSF Chimera rendering of SUMO1 6+. A) Solution NMR structure from pdb 1A5R, B) Last conformation after 1 µs simulation in explicit solvent C) Last conformation after 2 µs simulation in vacuum. Then CCS are calculated on gas phase conformations.

To investigate the variances highlighted in CIU heat maps (Fig. 5), we conducted a 250 ns MD simulation in implicit solvation (vacuum) on manually elongated proteins. This work provides a better understanding of high-energy conformations in CIU experiments.

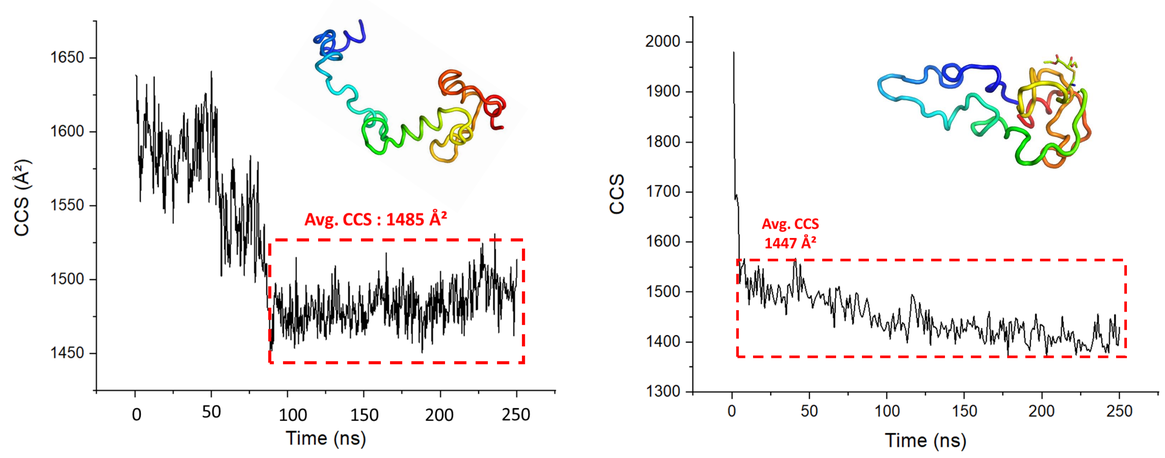

Figure 8: These figures represent the evolution of the CCS according to the MD time. On the left for SUMO1 and on the right, succ. SUMO1. We extract an averaged CCS when the protein is stabilized. CCS obtain this way are very close to those obtained in CIU experiments. This shows that proteins tend to adopt a more extended conformation but still compact after unfolding. Of course, snapshot represented here does not represent exact conformations after CIU.

Conclusions and perspectives

- The difference in CCS between SUMO1 and SUMO1+DEF corresponds to ***30 Å²***
- CIU experiments show that succination doesn't impact stability of SUMO1
  - ➡ But there is an inversion of population density at higher energies
- Molecular dynamics show us that proteins adopt a more unfolded but still compact conformation
  - ➡ New MD need to be done to understand why succination provokes these changes

Acknowledgments

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