May post-translational succination be involved in cardiac arrhythmia?
A joint study between (ion mobility) mass spectrometry and molecular dynamics

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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. But in some diseases, there is a germline mutation of the fumarase gene resulting in an enzymatic deficiency and therefore an accumulation of fumarate [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].

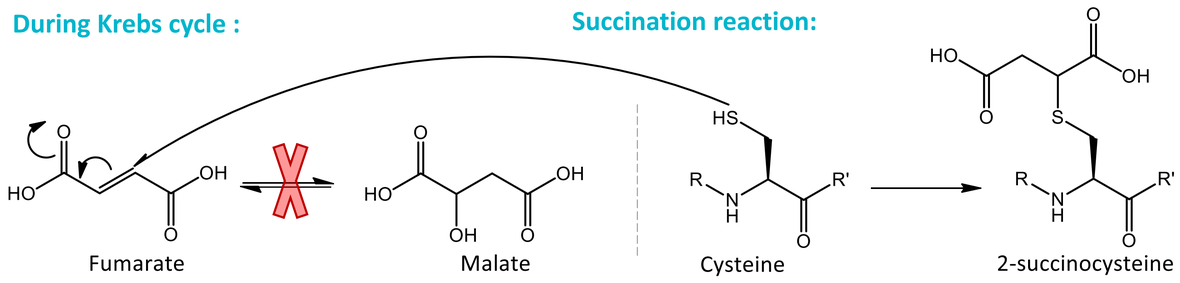


Figure 1: On the left, the deficiency of the hydration reaction catalyzed by the fumarate hydratase leads to an accumulation of fumarate. On the right the succination reaction between cysteine and the fumarate.

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 is involved in the stabilization of some proteins, including SERCA2a, a protein 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) and molecular dynamics whether the succination of SUMO1 has an impact on its 3D structure and therefore, whether it has an impact on its function (SUMOylation of SERCA2a) and induces cardiac arrhythmia.

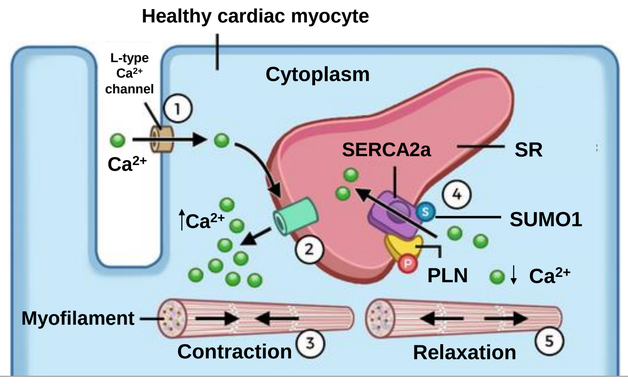


Figure 2: Scheme of SERCA2a function in the sarcoplasmic reticulum (SR) of cardiac myocytes. Upon a signal from a neurotransmitter, the SR releases Ca^{2+} ions into the cytoplasm, causing the heart to contract. These ions are then taken back by SERCA2a, a calcium-transporting ATPase, which allows the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

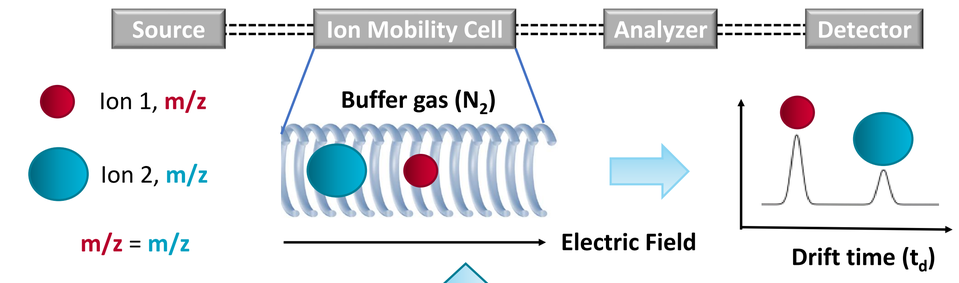
Goal of the study

GOAL

Determination of the collisional cross section (CCS) of proteins to evaluate their 3D structure
Before studying SUMO1, need for a model: **UBIQUITIN**

2

Experimental : Ion mobility mass spectrometry
WATERS SYNAPT G2-Si



Comparison between theoretical and experimental Collisional Cross Section (CCS)

Theoretical studies (workflow optimization)

1

Evaluation of the succination spontaneity
Liquid chromatography coupled to mass spectrometry

WATERS QToF API-US

1 Is succination a spontaneous process?

The first step in this study was to prove that the succination reaction can be spontaneous. Indeed, although this has been suggested in the literature [4], there is no direct evidence. To do so, experiments between Glutathione (GSH) and diethyl fumarate (DEF) were performed in vitro in ammonium acetate (pH 7 and 37°C). The reaction products were qualitatively studied by mass spectrometry to highlight the efficiency of the reaction, and LC-MS analyses were performed to obtain kinetic data.

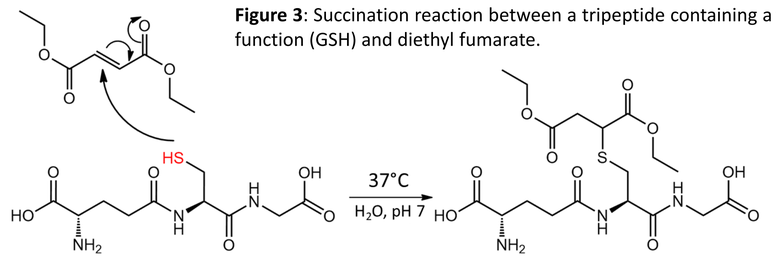


Figure 3: Succination reaction between a tripeptide containing a thiol function (GSH) and diethyl fumarate.

MS:

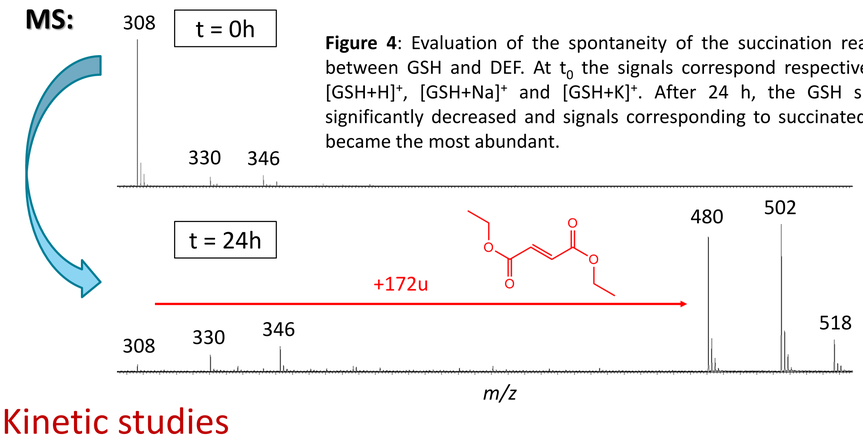


Figure 4: Evaluation of the spontaneity of the succination reaction between GSH and DEF. At t_0 the signals correspond respectively to $[GSH+H]^+$, $[GSH+Na]^+$ and $[GSH+K]^+$. After 24 h, the GSH signals significantly decreased and signals corresponding to succinated GSH became the most abundant.

Kinetic studies

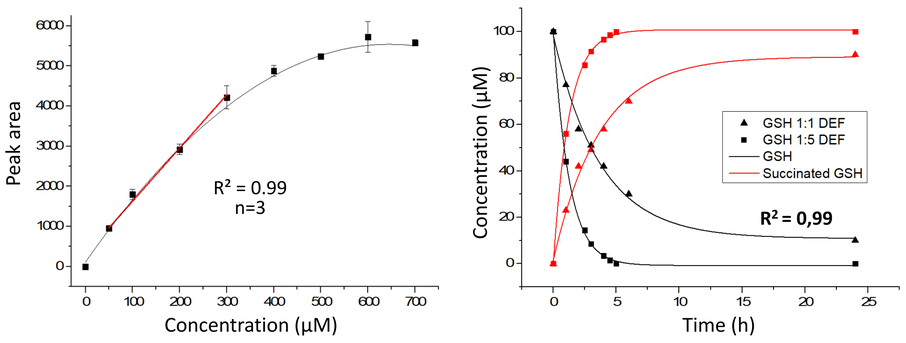


Figure 5: On the left: Evaluation of the Waters QToF API-US dynamic range for GSH. The linearity is preserved from 0 to 300 µM. On the right: Kinetic studies of the succination reaction at different molar ratios (5 mM). At a 1:1 ratio, the reaction reaches 90% conversion in 24h. At a 1:5 ratio the reaction reaches 100% conversion in 5h.

What about SUMO1?

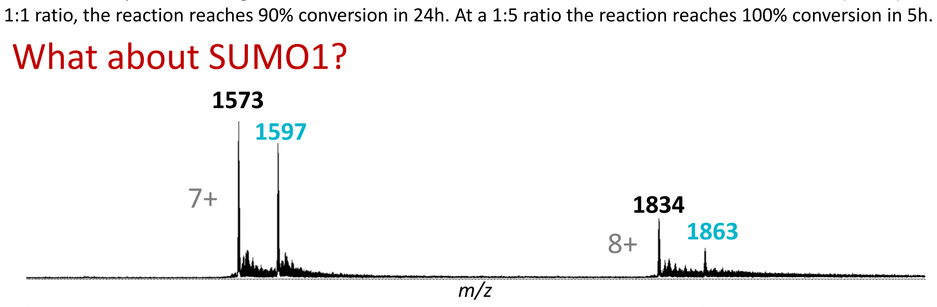


Figure 6: Native mass spectrometry of SUMO1 on SYNAPT G2-*Si* (positive mode). SUMO1 was incubated at 37°C (pH 7) for 24 hours with 10 molar equivalents of diethyl fumarate. The black-labeled signals correspond to the SUMO1 protein, and the blue-labeled ones represent the succinated SUMO1.

2 How evaluate the 3D structure of proteins?

Theoretical studies

To get structural information, the theoretical methodology was optimized to simulate as correctly as possible the protein structure in Ion Mobility experiments and then to calculate theoretical CCS to compare to experimental results. The ubiquitin 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 ff14SB force-field for the protein and the TIP3P 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) [5]. This method is currently the most accurate to compute CCS and compare them to the CCS_{exp}.

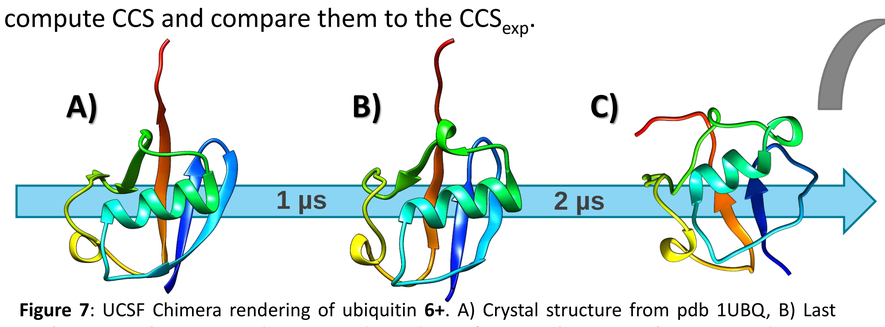


Figure 7: UCSF Chimera rendering of ubiquitin **6+**. A) Crystal structure from pdb 1UBQ, B) Last conformation after 1 µs simulation in explicit solvent C) Last conformation after 2 µs simulation in vacuum.

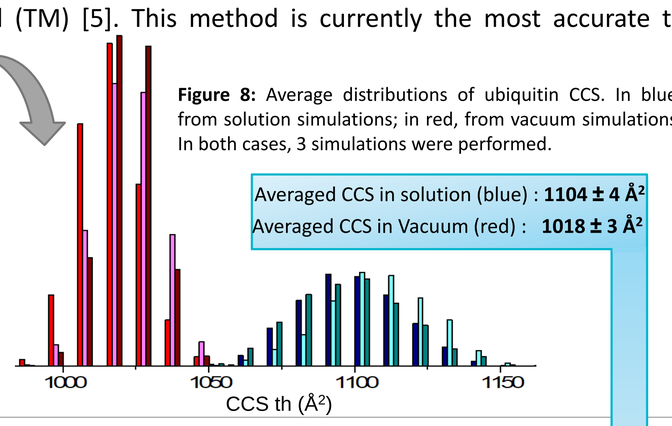


Figure 8: Average distributions of ubiquitin CCS. In blue, from solution simulations; in red, from vacuum simulations. In both cases, 3 simulations were performed.

Experimental studies

CCS were measured on a Waters Synapt G2-S*i* (Travelling Wave Ion Mobility Cell, TWIMS) by using our CCS calibration based on polymer CCS measured on a linear drift tube in He [6]:

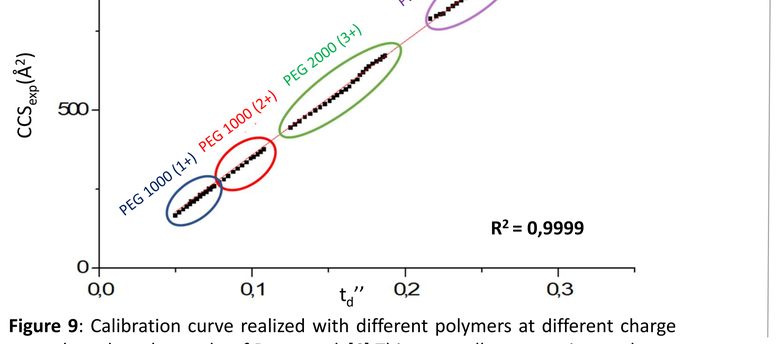


Figure 9: Calibration curve realized with different polymers at different charge states based on the works of Duez *et al.* [6] This curve allows us to interpolate a CCS based on a measured/calculated t_d'' for ubiquitin.

Mason – Schamp equation adapted for TWIMS:

$$\Omega = \frac{\sqrt{18\pi}}{16}\frac{ze}{\sqrt{k_B T}}\sqrt{\frac{1}{m_I}+\frac{1}{m_N}}\frac{760}{P}\frac{T}{273{,}2}\frac{1}{N}\,A\,t_D^B$$

Ω_{ref} = A' td'' = **3592 t_d''**

Experimental CCS: **1028 ± 29 Å²**

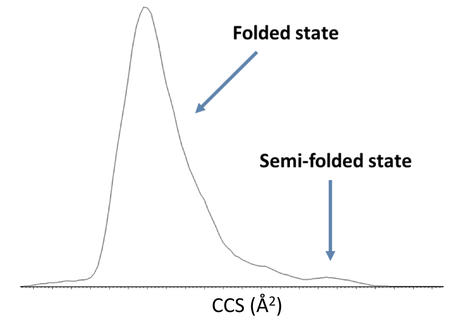


Figure 10: CCS distribution recorded for Ubiquitin 6+ under native conditions. The most intense signal corresponds to the native structure, the weakest one to a semi-folded structure, occurring despite soft conditions.

Conclusions

In this study, we evaluate the spontaneity of succination of free thiol functions to ultimately assess whether this reaction can have an impact on the 3D structure of SUMO1, SERCa2A, and finally on cardiac arrhythmia. The first aspect of the study is therefore to evaluate the spontaneity of the reaction. Kinetic studies show that the reaction is spontaneous and even almost complete at 37°C for 24 hours under physiological conditions for the GSH tripeptide. The second part consists in developing a procedure to study theoretical modifications of the 3D structure of proteins after succination. The above results show the efficiency of the theoretical workflow through a very good fit between experimental and theoretical CCS. The next step of this study is to extend the experimental et theoretical investigations on SUMO1. It is also planned to study structural modifications in solution by using circular dichroism.

Acknowledgements

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References

[1] S. C. Fletcher *et al.*; *Biochem. Soc. Trans.*, **48**, 1843–1858, (2020)
[2] N. Frizzell *et al.*; *J. Biol. Chem.*, **38**, 25772–25781, (2009)
[3] C. Kho *et al.*; *Nature*, **477**, 601–606, (2011)
[4] N. L. Alderson *et al.; Arch. Biochem. Biophys.*, **450**, 1–8, (2006)
[5] S. A. Ewing *et al.; J. Am. Soc. Mass Spectrom.*, **284**, 587–596, (2017)
[6] Q. Duez *et al.*, *J. Am. Soc. Mass Spectrom.*, **28**, 2483–2491, (2017)

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