MP 041 Intact Protein MALDI Mass Spectrometry Imaging (MSI) to characterize target engagement and blood-brain barrier penetration for amyotrophic lateral sclerosis (ALS)

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OVERVIEW

- Target engagement and exposure at the site of action are critical to link protein modification to pharmacodynamics.
- Covalent drugs bind irreversibly to target proteins, which gives an opportunity to use intact protein mass spectrometry methods to detect the covalent drug-protein complex.
- Fluoroscein-5-maleimide (F5M) was used as a chemical probe for proof-of-concept in ALS mouse brain homogenate using intact protein MALDI mass spectrometry.
- Our *in vivo* investigation involved direct detection of drug-protein complex *in situ.* The drug candidate was *S*-XL6. This prototype cyclic thiosulfinate was dosed into ALS mice and intact protein MALDI MSI was used to visualize the interaction of S-XL6 with its target, copper zinc superoxide dismutase 1 (SOD1). **This workflow informs target engagement and blood-brain barrier (BBB) penetration and unlike conventional bioanalysis, also preserves the spatial component.**
- Direct detection of drug-protein complexes can enable on and off-target identification in drug development and coupling to MSI enables visualization at the site of action.

INTRODUCTION

- ALS is a fatal, neurodegenerative disease characterized by death of upper and lower motor neurons in brain and spinal cord. Drugs must therefore cross the BBB.
- Mutations in SOD1 protein are linked to familial ALS (fALS), associated with loss of quaternary structure, protein destabilization and aggregation.
- Dimer stabilization can provide as a potential therapeutic strategy for ALS.

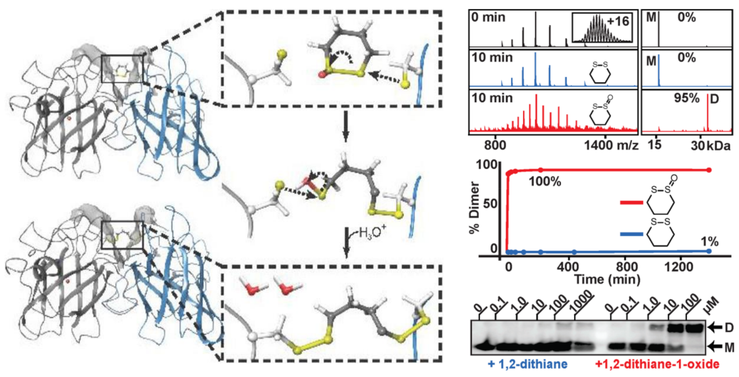


Figure 1. Cyclic thiosulfinate cross-link SOD1 at Cys111 to form a dimer in vitro.[1]

METHODS

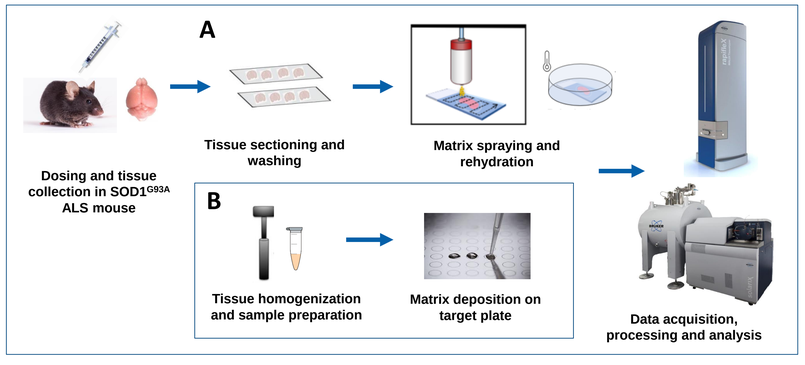
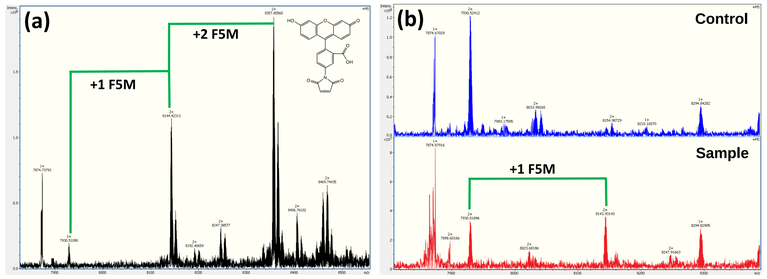
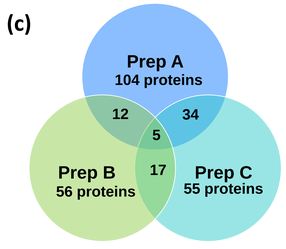


Figure 2. Schematic diagram of MALDI workflow for detection of intact proteins. (A) MALDI MSI workflow for detection of intact proteins from ALS mouse brain sections. (B) Workflow for detection of intact proteins from mouse brain homogenate.

RESULTS





Sample Preparation	Total proteins detected	Total proteins modified by probe detected
Prep A	104	6
Prep B	56	5
Prep C	55	2

Prep A: *Protein precipitation using chloroform methanol water + re-solubilization by 80% formic acid*
Prep B: *Protein precipitation using chloroform methanol*
Prep C: *Protein concentration by ultrafiltration*

Figure 3. MALDI FTICR MS of intact proteins. (a) Purified fALS variant SOD1^{G93A} modified by chemical probe F5M. (b) Control vs. sample ALS mouse brain homogenate spiked with F5M, showing **target (SOD1^{G93A}) engagement *in vitro*.** (c) Off-target analysis. To develop methods three different sample preparations were tested and probe-modified proteins were detected in ALS mouse brain homogenate. **An example of off-target was 9772 m/z, putatively identified as High affinity cationic amino acid transporter 1.**

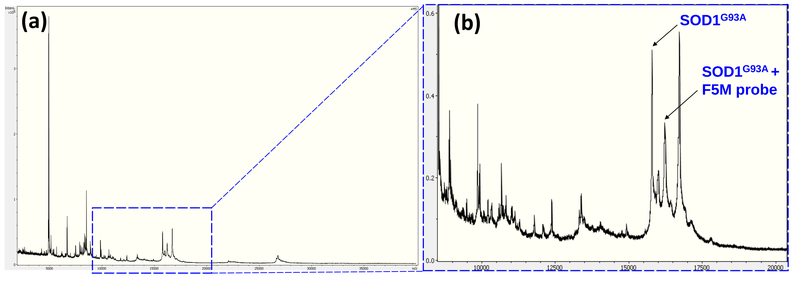


Figure 4. MALDI TOF MS of intact proteins *in vitro* (spiked mouse brain homogenates). The average spectrum of the entire TOF MS data set is shown in (a). Expanding the intensity scale (b) highlights the **target engagement of SOD1^{G93A}** using chemical probe F5M *in vitro*.

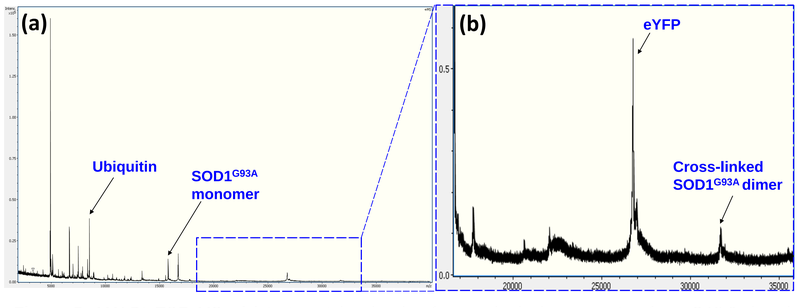


Figure 5. MALDI TOF MS of intact proteins *in vivo* (mouse brain homogenates from *S*-XL6 dosed animals). The average spectrum of the entire TOF MS data set is shown in (a). Expanding the intensity scale (b) highlights the detection over high m/z range. Arrows indicate (1) Ubiquitin signal (m/z 8540), (2) SOD1^{G93A} monomer (m/z 15858), (3) eYFP (m/z 26774), **(4) Cross-linked SOD1^{G93A} dimer (m/z 31836)**. Note that we are using transgenic G93A mice bred with mice expressing eYFP in specific cells, so that motor neurons can be identified in MALDI MSI.[2]

RESULTS

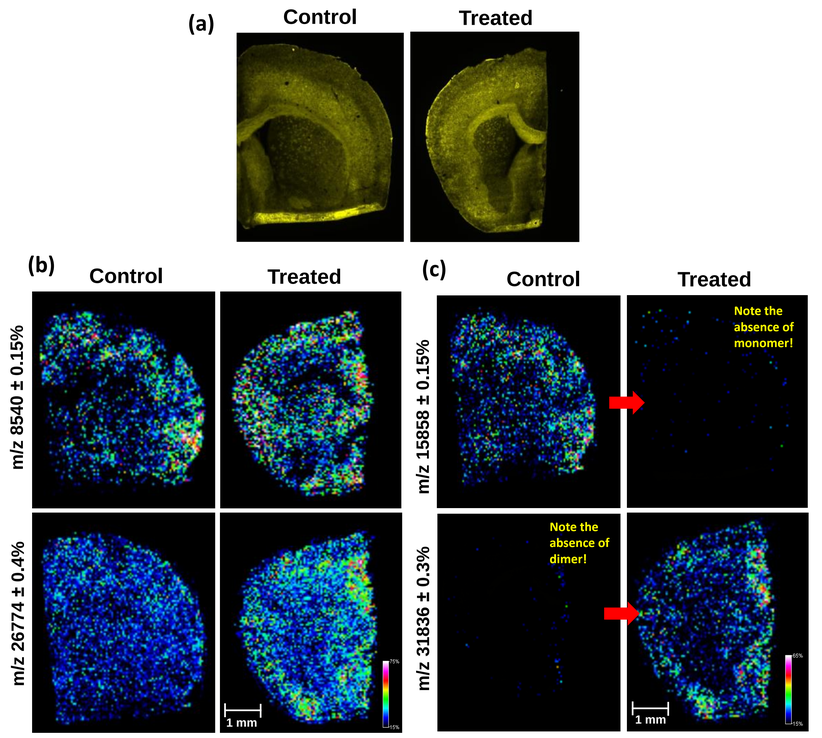


Figure 6. MSI at 50 μm spatial resolution showing blood-brain barrier penetration and target engagement of S-XL6 *in vivo*. Control and treated brain section fluorescent images are shown in (a), MALDI MSI images on intact proteins Ubiquitin (m/z 8540) and eYFP (m/z 26774) are shown in (b). **MALDI MSI showing blood-brain barrier penetration of *S*-XL6 shown in (c), detecting SOD1^{G93A} monomer (m/z 15858) and cross-linked SOD1^{G93A} dimer (m/z 31836). This result (c) is consistent with the MOA of *S*-XL6.**

CONCLUSION

We demonstrated
- Intact protein MS to define target engagement *in vivo*.
- Intact protein MS to define off-target binding *in vitro*.
- Intact protein MSI to define target engagement and BBB *in vivo*.

Future studies are aimed at increasing the sensitivity (number of proteins detected) and identifying the m/z's corresponding to off-target binding.

REFERENCES

1. Donnelly D. et al, (2018) *Journal of American Chemical Society 140 (20)*, 7377-7380
2. Schmitt N. D. et al, (2019) *Analytical Chemistry 91 (6)*, 3810-3817

ACKNOWLEDGEMENTS

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