Speciation and Cellular Activity of Manganese Superoxide Dismutase Mimic

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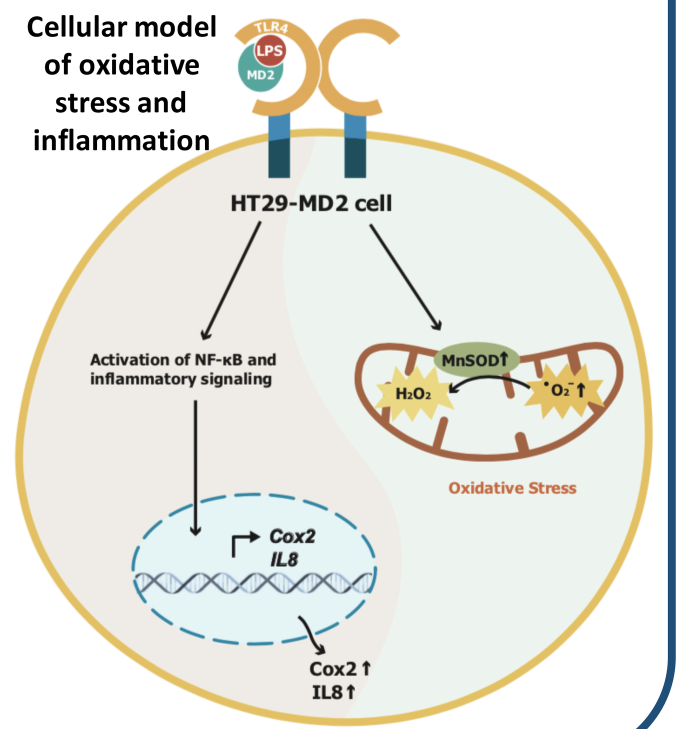
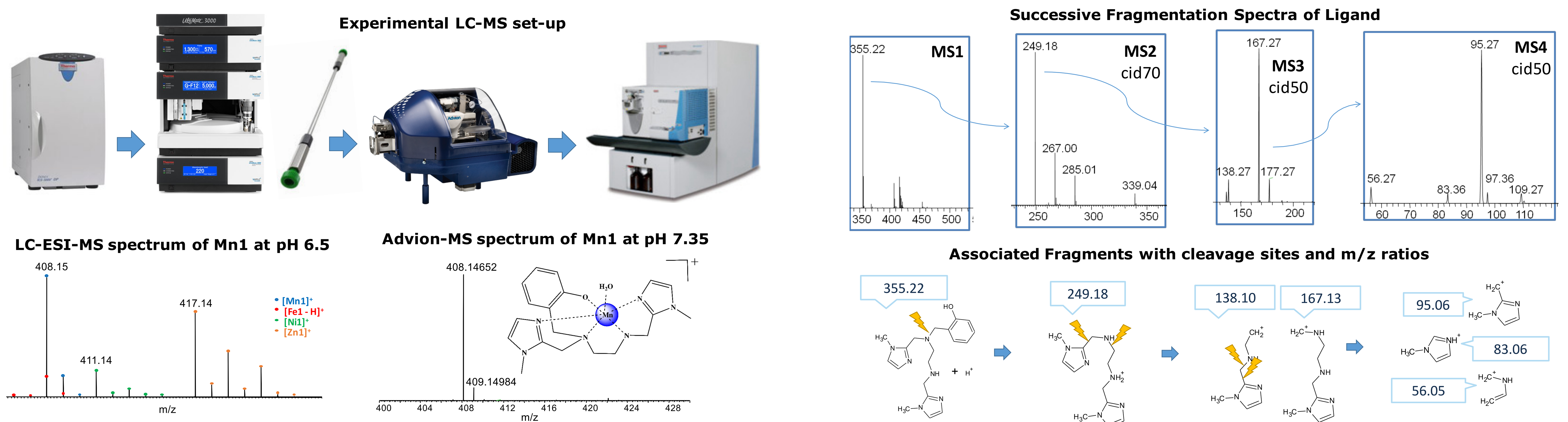
Abstract

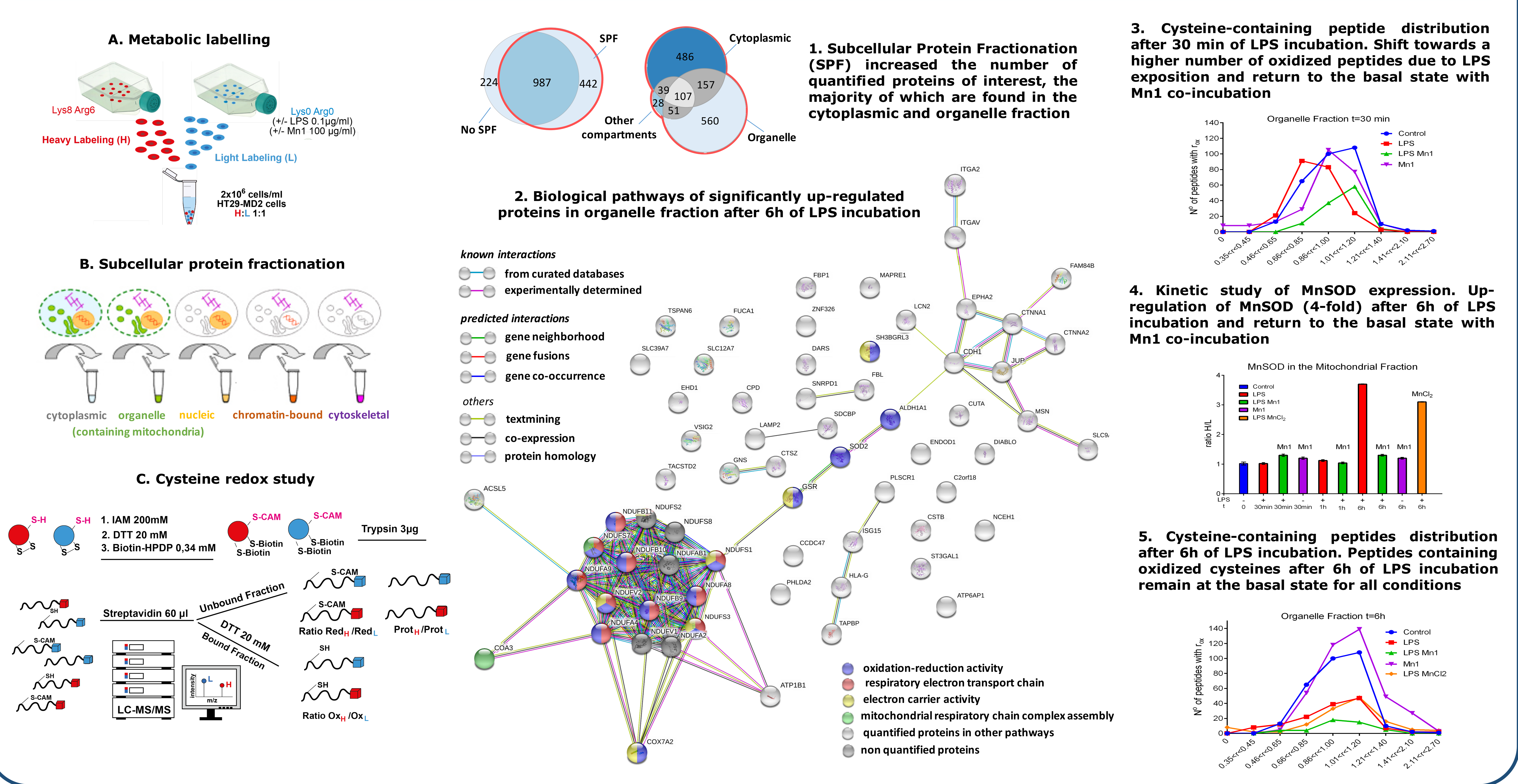
Superoxide Dismutases (SODs) are metalloenzymes involved in the cellular antioxidant protection pathway controlling reactive oxygen species (ROS). However, the SOD activity is weakened in intestinal epithelial cells from patients suffering from inflammatory bowel diseases (IBDs), leading to an increase in ROS. To complement for this SOD deficiency, we have investigated the effect of the manganese complex Mn1, an SOD mimic with intracellular anti-superoxide and anti-inflammatory activity, on the protein level of intestinal LPS-stressed epithelial cells (HT29-MD2), as a model of oxidative stress and inflammation, using bottom-up redox proteomics. With a differential labelling approach coupled to a SILAC (Stable Isotope Labelling by Amino acids in Cell culture) quantification, we quantified the changes in protein expression, as well as the oxidation state of cysteines, the main targets of protein oxidation. Subcellular protein fractionation increased the number of quantified proteins by 15%, especially from organelles such as mitochondria, crucial for this study and otherwise difficult to detect. A kinetic study (30 min to 6 h +/- LPS/Mn1) highlighted an increased number of oxidized peptides in the organelle fraction, after a 30 min LPS treatment, that returned to the basal state after 6 h. These results are in agreement with an up-and down-regulation of proteins involved in the respiratory and electron transport chain after a 30 min LPS incubation, and an up-regulation of proteins involved in the regulation of oxidative stress (such as MnSOD), after 6 h. The co-incubation of LPS with Mn1 reduced the increased number of oxidized peptides and prevented the up-regulation of MnSOD (after 6 h), mimicking its antioxidant action. In parallel, in order to detect Mn1 (small hydrophilic molecule) inside cells and to understand its metabolization and speciation intracellularly we characterized the spectra of the Mn1 complex and its ligand in a non cell environment, using hydrophilic interaction chromatography and MS/MS fragmentation. We managed to separate Mn1 from the different chelated forms of the ligand with metal ions such as Fe^{2+}, Zn^{2+} and Ni^{2+} and obtained clean MS signature spectra.



Mn1 Speciation



Mn1 Effect on the cellular redoxome



Conclusions and perspectives

The Mn1 SOD mimic reduced the increased number of oxidized peptides in intestinal LPS-stressed epithelial cells (HT29-MD2) after 30 min and prevented the up-regulation of MnSOD by efficiently restoring the oxidation levels after 6h. We managed to obtain clean MS signature spectra of Mn1 and further experiments are on the way to correlate the proteomic data with the *in cellulo* metabolome and speciation of Mn1.

References

(1) Bernard A-S, *Dalton Trans*., **2012**, 41, 6399 , (2) Mathieu E. et al, *Inorg. Chem*, **2017**, 56 5, 2555

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