

# Single-Cell Metabolite Annotation by Tandem Mass Spectrometry and *Ab Initio* Molecular Dynamics

**Mateo Topalović,<sup>a</sup> Ivana Marković,<sup>b,c</sup> Vlatka Periša,<sup>b,c</sup> Maja Lukić,<sup>b,c</sup> Ema Pavičić,<sup>d</sup> Iva Lukić,<sup>b,c</sup>  
Stefan Mrđenović,<sup>b,c</sup> Igor Lukačević<sup>a</sup> and Željko Debeljak<sup>b,c</sup>**

*mtopalovic@fizika.unios.hr*

<sup>a</sup>*Josip Juraj Strossmayer University of Osijek, Department of Physics, Trg Ljudevita Gaja 6, 31000 Osijek, Croatia*

<sup>b</sup>*University Hospital Centre Osijek, Josipa Huttlera 4, 31000 Osijek, Croatia*

<sup>c</sup>*Josip Juraj Strossmayer University of Osijek, Faculty of Medicine, Josipa Huttlera 4, 31000 Osijek, Croatia*

<sup>d</sup>*Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 18, 31000 Osijek, Croatia*

Accurate single-cell mass spectrometry (MS) imaging and metabolite identification remain challenging due to the small size of cells, the limited quantity of desorbed material, and the high density of  $m/z$  values in metabolite databases. A highly sensitive analytical approach that enables metabolite annotation by matching  $m/z$  values from single-cell MS/MS (tandem mass spectrometry) spectra with fragment  $m/z$  values calculated using *ab initio* molecular dynamics was developed.

This method was applied to annotate a signal at  $m/z$  337.11 Da, which is elevated in chronic lymphocytic leukemia [1]. Five candidate compounds were selected through database searching. Prior to single-cell MS/MS imaging, the procedure was conducted on S-nitrosoglutathione (GSNO), one of the candidates. Five fragment ions were consistently observed in both experimental and *in silico* spectra of the GSNO solution.

After optimizing the sensitivity of single-cell MS imaging, MS/MS spectra were acquired from individual lymphocytes. These spectra revealed 0–3 fragment matches with *in silico* spectra of three glutathione-related compounds. In contrast, no correspondence was found between experimental and calculated spectra for the remaining candidate compounds.

## References:

- [1] I. Marković, Ž. Debeljak, B. Dobrošević, M. Lukić, S. Mrđenović, A. Kotris, B. Bošnjak, B. Dmitrović, *Clin. Chim. Acta.* **561** (2024) 119758.