4D-Lipidomics investigation of in *C. elegans* daf-2 mutants related to ageing and longevity

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The gene daf-2 was one of the first genes described to extend the lifespan in the model organism Caenorhabditis elegans. daf-2 encodes for the sole homolouge of the insulin-like growth factor 1 (IGF-1) receptor in C. elegans. daf-2 mutants show different metabolic adaptations, including changes in lipid metabolism. Here, a 4D-Lipidomics workflow was applied to investigate characteristic changes in the complex lipidome of C. elegans wildtype vs. daf-2 mutants. 4D stands for the 4 analytical information obtained from the LC-MS/MS experiments: 1. Retention time 2. m/z values 3. MS/MS spectra 4. CCS values (collisional cross section) from ion mobility measurements.

Comprehensive coverage of detected lipids with a corresponding MS/MS spectrum is required for confident lipidome characterization. With the timsTOF Pro mass spectrometer (Bruker) utilized here, this is realized by the unique PASEF (Parallel Accumulation Serial Fragmentation) acquisition mode. This scan mode offers the possibility to generate clean MS/MS spectra by trapped ion mobility separation (TIMS) of chromatographically non-resolved isobaric lipids at high acquisition speeds. Furthermore the ion mobility measurements add an analytical dimension, the so called CCS values (collision cross section). These values can be used for the identification of a compound in addition to traditional identification criteria.

An integrated workflow for evaluating 4D-Lipidomcics data will be presented. Comparing lipid extracts from C. elegans wild type and mutants enabled the pinpointing of characteristic lipids and their confident identification. Merging information from PASEF MS/MS spectra acquired in positive and negative mode provided complementary information on lipid headgroups and fatty acid side chains. By matching measured CCS values to predicted values increased confidence in lipid assignment even further.