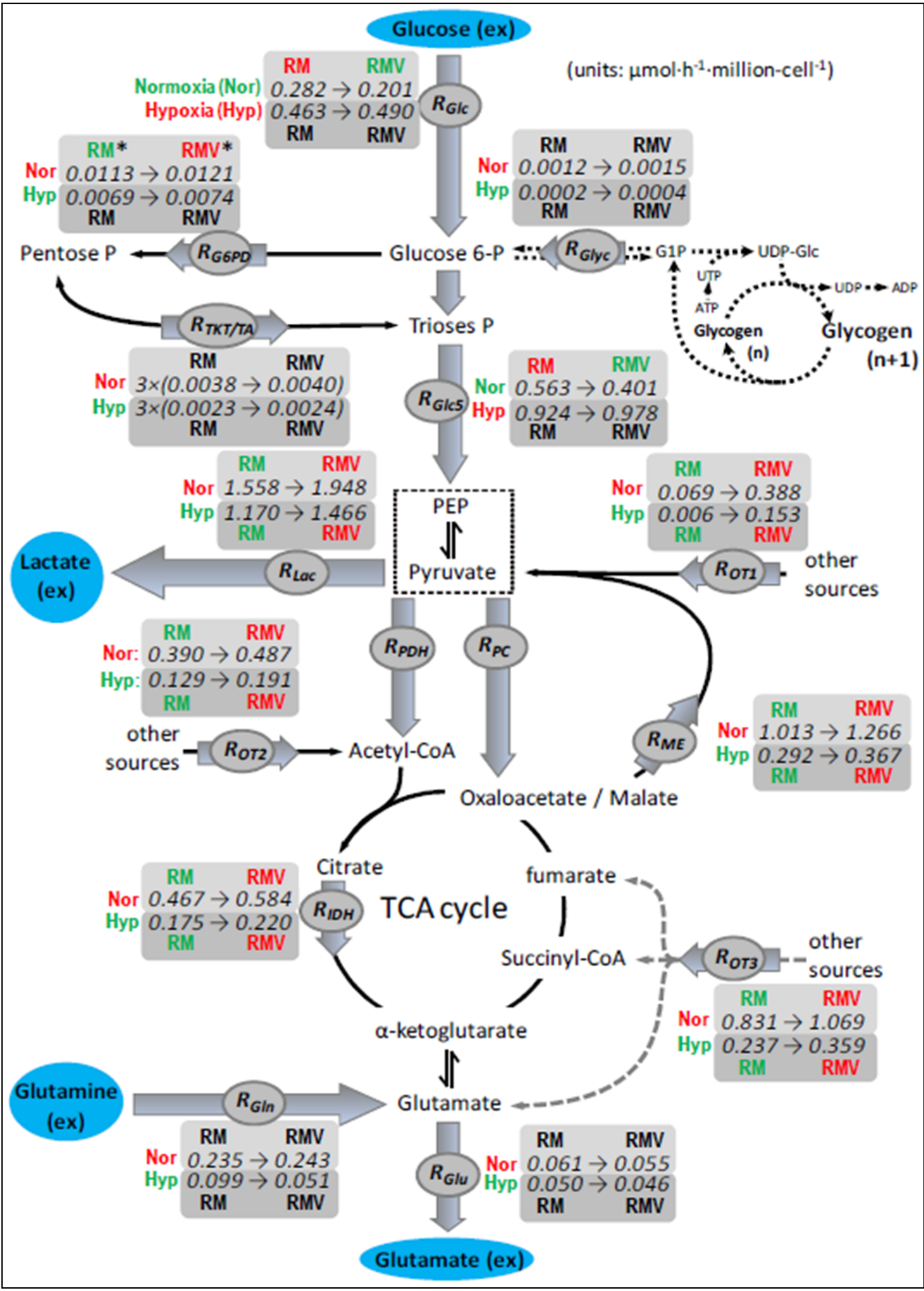
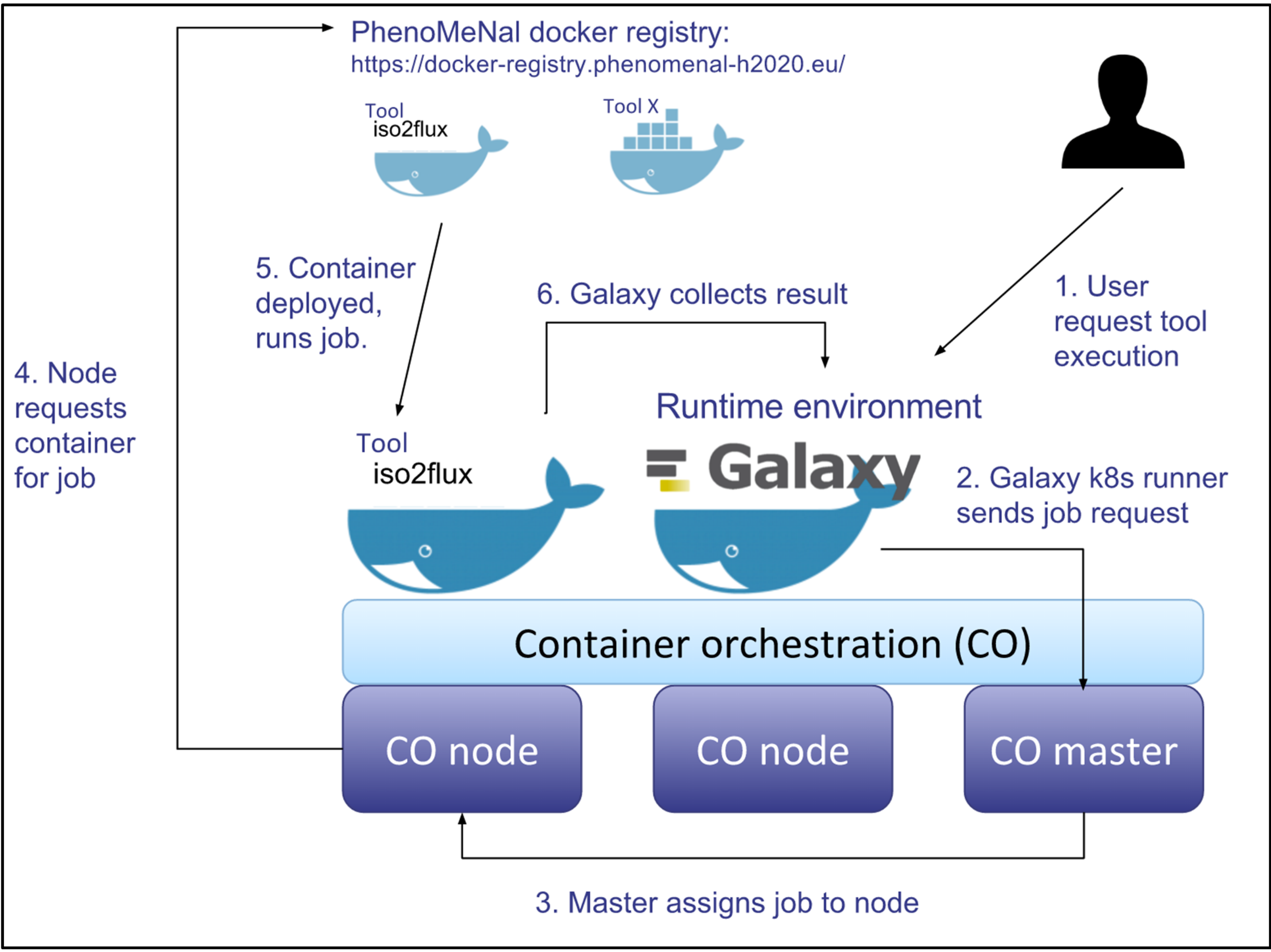




Workflows for fluxomics in the framework of PhenoMeNa project

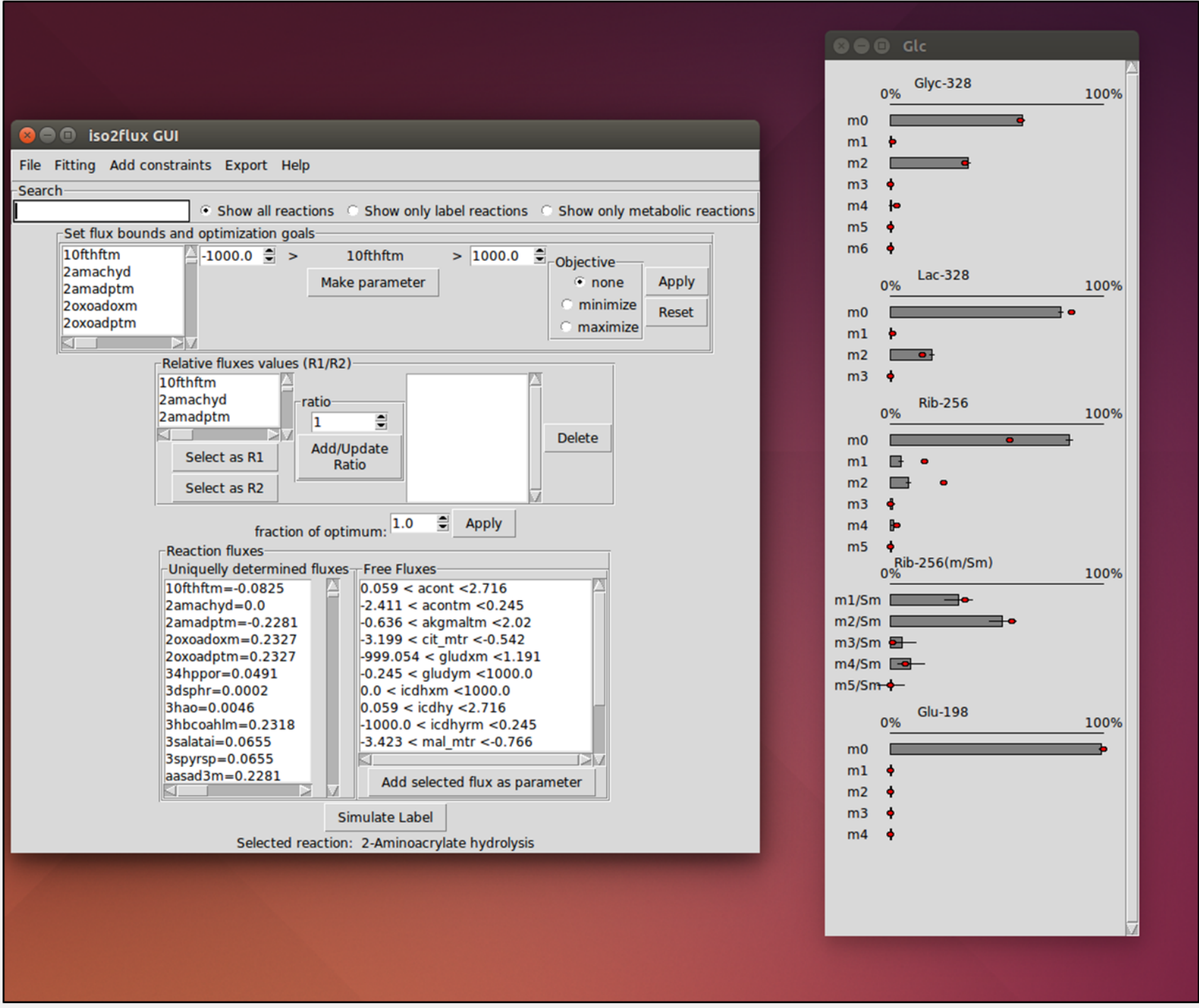
Hypotheses regarding intracellular fluxes can be evaluated by comparing measured and predicted label (e.g. ¹³C) isotopologue (mass isotopomer) distributions. Assuming a metabolic network of biological reactions, the computational estimation of metabolic fluxes is based on measured labeling patterns in intracellular metabolites resulting from metabolizing labeled substrates, frequently together with measured metabolic consumption and production rates.

In the framework of the PhenoMeNa project and e-infrastructures (www.phenomenal-h2020.eu/home/), two workflows were prepared using previously developed programs for analysis of intracellular fluxes from isotopologue distributions. With this aim, programs are adapted to the workflows, docker container dockerfiles for each program are uploaded to GitHub repositories, added to Jenkins for building the docker images and Galaxy wrappers are written for these tools. Isotopologue distributions will be available on data repositories in MetaboLights (www.ebi.ac.uk/metabolights). The workflows under preparation include two of the following programs: 1) **Midcor**, an R-tool for primary ¹³C data correction for natural isotope enrichment; 2) **Iso2Flux**, a Python-tool for steady state analysis of fluxes based on measured distributions of isotopologues; 3) **Isodyn**, a C-tool for dynamical analysis of fluxes based on time-series measured distributions of isotopologues. A preliminary version of the workflow including Midcor and Iso2Flux has been already tested. First, Midcor reads and corrects ¹³C data and then Iso2Flux perform the analysis of flux distributions.



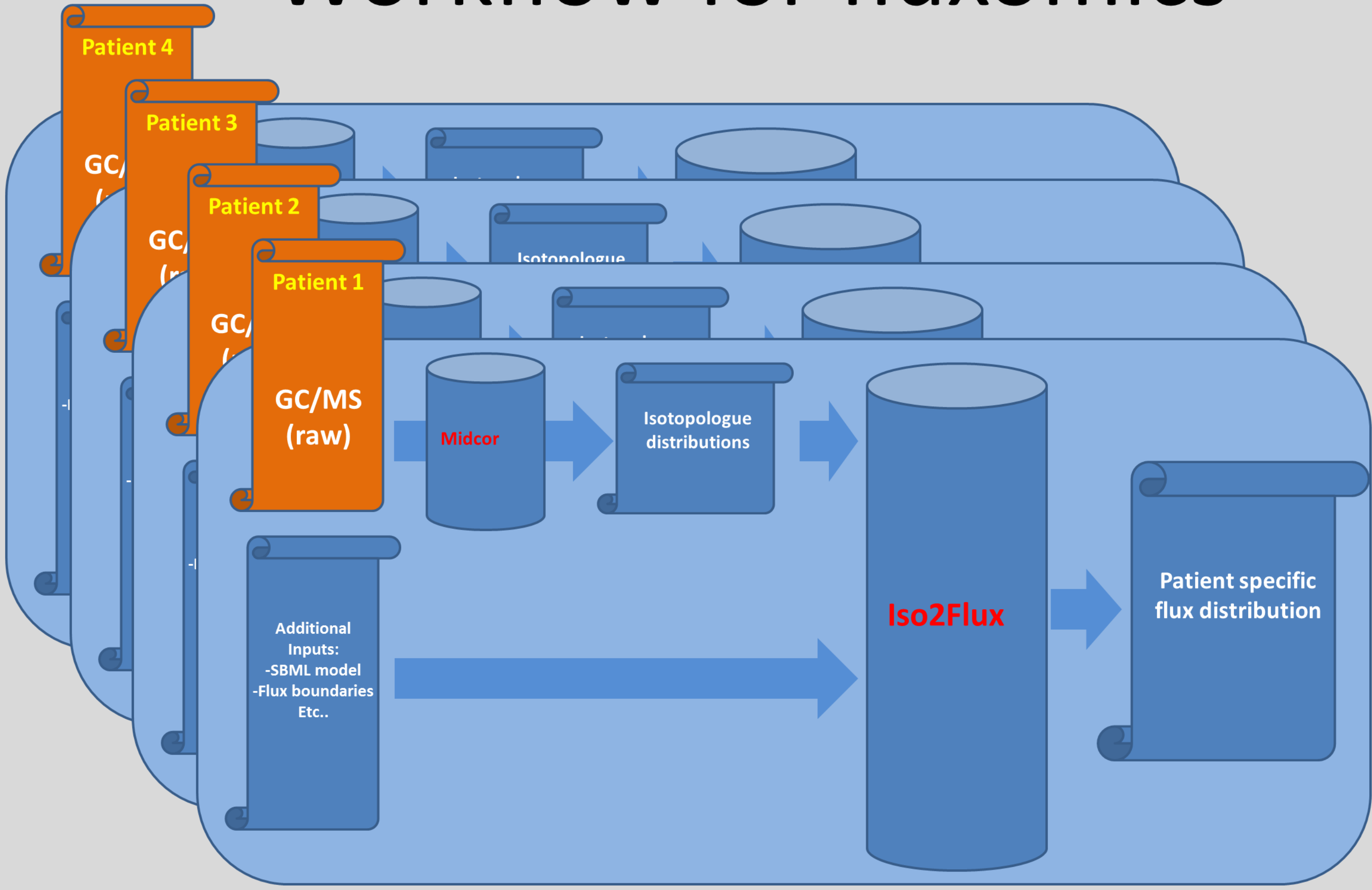
Adjusted flux distributions. The ¹³C flux analysis shows the flux distributions in the central carbon metabolism of Human Umbilical Vein Endothelial Cells (HUVECs) in the presence of VEGF, under normoxia and hypoxia. Fluxes are predicted with the ¹³C tracer analysis encompassing ¹³C label enrichment analysis and the concentrations of the consumption/production of metabolites measured. The flux distributions are represented as the reactions (R) covering the central carbon metabolism. Nor – Normoxia, Hyp – Hypoxia, RM – HUVECs in restricted medium (control); RMV – HUVECs with RM+VEGF, IDH – isocitrate dehydrogenase, Lac – lactate, G6PD – glucose-6-phosphate dehydrogenase, Glc – glucose, Gln – glutamine, Glu – glutamate, Glyc – glycogen, ME – mitochondrial reaction flux, OT1, OT2, OT3 – group of reactions with alternative sources of carbons, PC – pyruvate carboxylase. PDH – pyruvate dehydrogenase, TKT/TA – transketolase/transaldolase. Red colour denotes increase and green colour denotes decrease in fluxes in the respective conditions.

Graphical-supported detailed analysis



Repeated analysis of large data collections. Iso2Flux can be operated either through an intuitive graphical interface (shown above) or as a command line program. Although lot of data can be available, a serious analysis of the data always requires the iterative validation of an expert biochemist, for example to decide the scope of the analysed network, including or not the different metabolic pathways (glycolysis, Krebs cycle, ...). Then, looking for repeated patterns, much time will be required for the repeated analysis required by large data collections, like for clinical data. A detailed analysis using the graphical interface will be required to just one case. This preliminary analysis will be used to target the analysis to the reduced set of fluxes or ratios among fluxes showing changes. From this preliminary analysis the conditions for an automatic and fast analysis of the rest of cases will be established using Midcor and Iso2Flux as components of a workflow for fluxomics under the Galaxy environment.

Workflow for fluxomics



Iso2Flux will also be usable as part of the fluxomics workflow of the PhenoMeNa project. With this aim, a dockerfile for Iso2Flux has been uploaded to the GitHub repository of PhenoMeNa allowing to automatically build a docker image capable of running Iso2Flux. A Galaxy wrapper for this docker image is used to integrate Iso2Flux into the fluxomics workflow of PhenoMeNa. Specifically, IsoFlux will be using the output of Midcor, an R-tool for experimental ¹³C data correction for natural isotope enrichment, as well as additional inputs to generate a set of metabolic flux predictions. Once completed, the fluxomics workflow of PhenoMeNa will allow to read data collections from repositories like MetaboLights (www.ebi.ac.uk/metabolights) and estimate repeated patterns of specific metabolic flux distributions.

