Integrative omics for the discovery of biosynthetic pathways using MEANtools (MEtabolite ANticipation tools)

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Introduction

MEANtools is a **python-based** workflow that integrates genomic, transcriptomic, and metabolomic data with enzymatic reaction databases to predict metabolic pathways, by identifying mass differences between metabolites that are co-abundant with transcripts whose enzymatic products are capable of catalysing biosynthetic reactions.
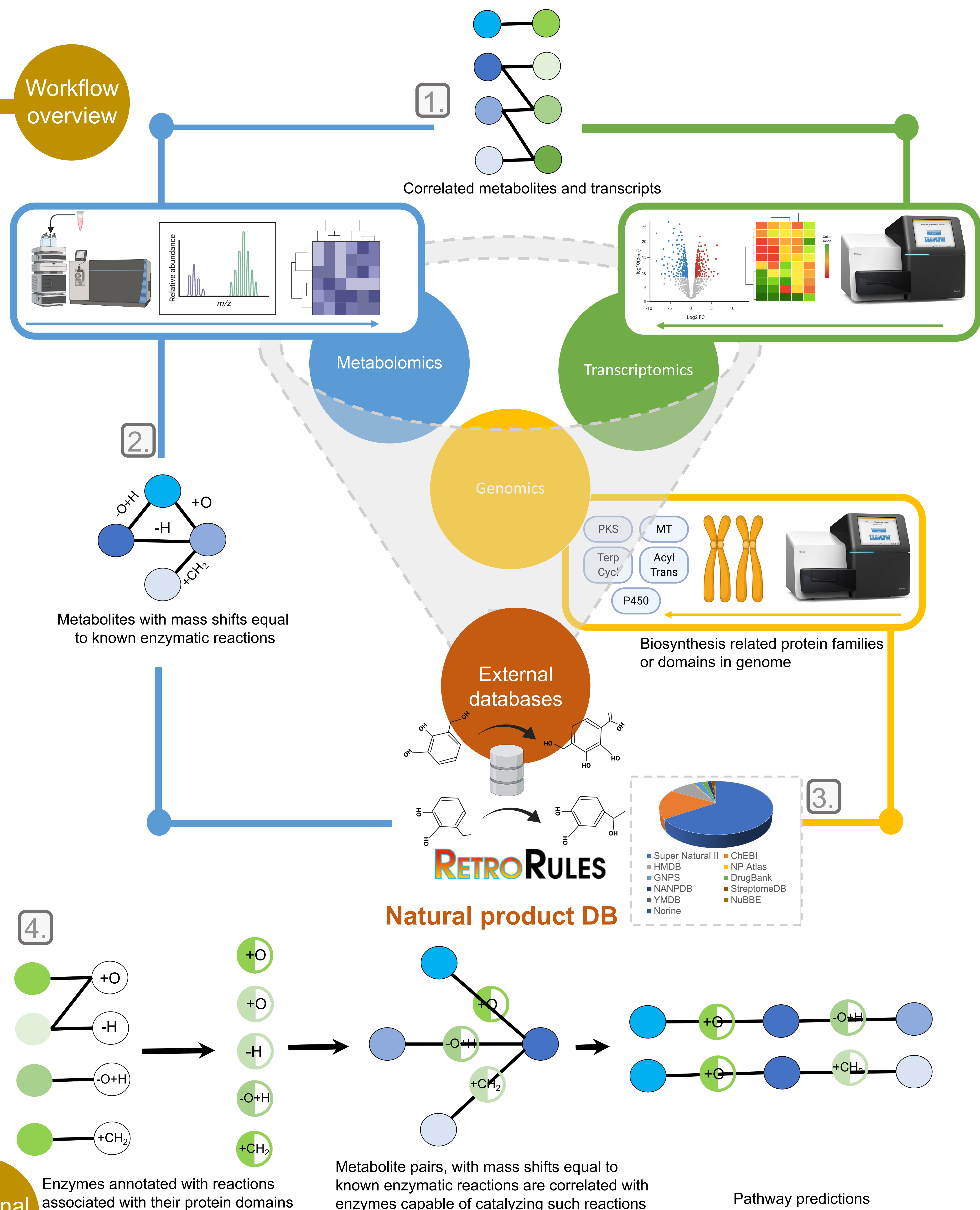
MEANtools first identifies strongly correlated transcript-metabolite pairs and then annotates these pairs with reactions by querying the **RetroRules** (Duigou *et al.* 2019) and **Natural Product** (Stokman *et al.* Msc. thesis WUR) databases. The annotated pairs represent an enzyme-encoding transcript with a protein domain capable of catalysing an enzymatic reaction that has a correlated metabolite as a substrate or product.

To further expand the chemical transformation search space of MEANtools, the RetroRules database has been further expanded by including **KEGG** (Kyoto Encyclopedia of Genes and Genomes) orthology (Nakaya *et al.* 2013) and **Rhea** (Alcantara *et al.* 2012) reaction databases.

https://git.wur.nl/medema-group/meantools.git

1. Correlations are computed between transcripts and metabolites based on gene expression and metabolite abundances.

2. Mass signatures from the metabolomic data are converted to Molecular Families (MF). MFs are then mapped with enzymatic reaction databases to identify pairs with mass differences associated with known enzymatic reactions.

3. Protein families/domains encoded by the genes are used to query enzymatic reaction databases.

4. MEANtools then identify cases in which metabolite pairs are correlated to a transcript that encodes an enzyme capable of catalyzing a reaction that explains their mutual mass difference.

5. The product of these reactions are then mapped to other mass signatures in the metabolome and the procedure is then repeated multiple times to generate pathway predictions.
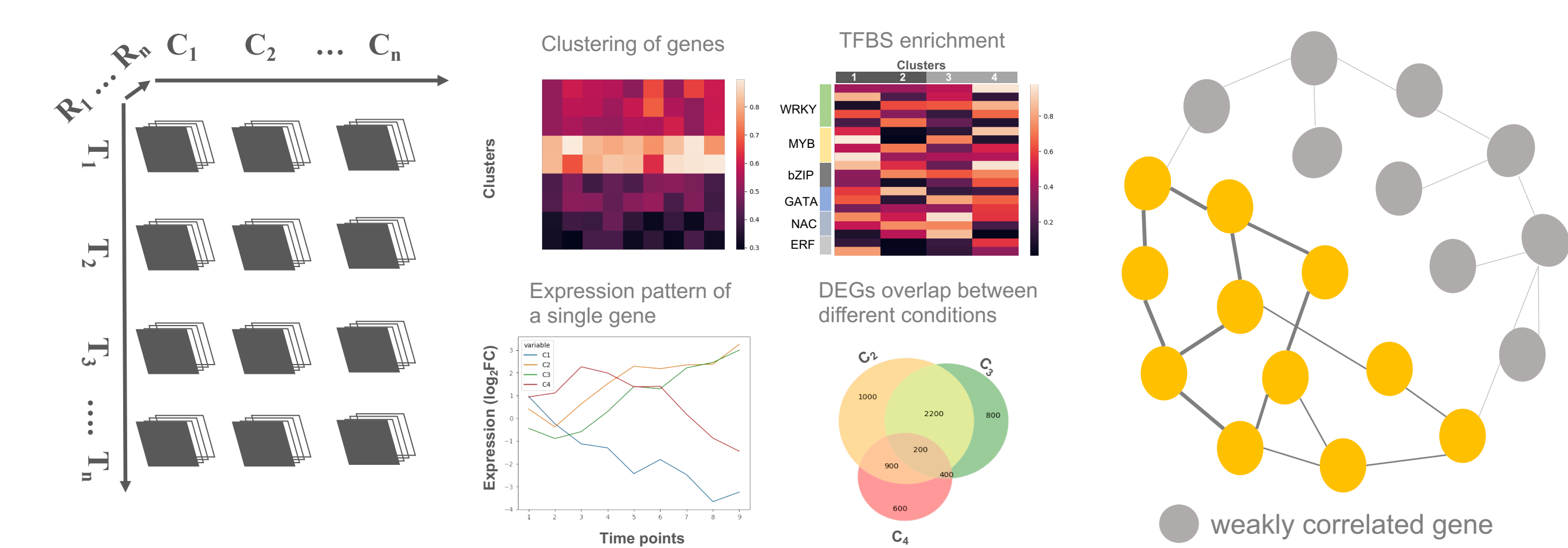
Workflow overview

1. Correlated metabolites and transcripts

Metabolomics

Transcriptomics

Genomics

2. Metabolites with mass shifts equal to known enzymatic reactions

External databases

PKS   MT
Terp Cycl   Acyl Trans
P450

Biosynthesis related protein families or domains in genome

RETRORULES
Natural product DB

3.
■ Super Natural II   ■ ChEBI
■ HMDB   ■ NP Atlas
■ GNPS   ■ DrugBank
■ NANPDB   ■ StreptomeDB
■ YMDB   ■ NuBBE
■ Norine

4. Enzymes annotated with reactions associated with their protein domains

Metabolite pairs, with mass shifts equal to known enzymatic reactions are correlated with enzymes capable of catalyzing such reactions
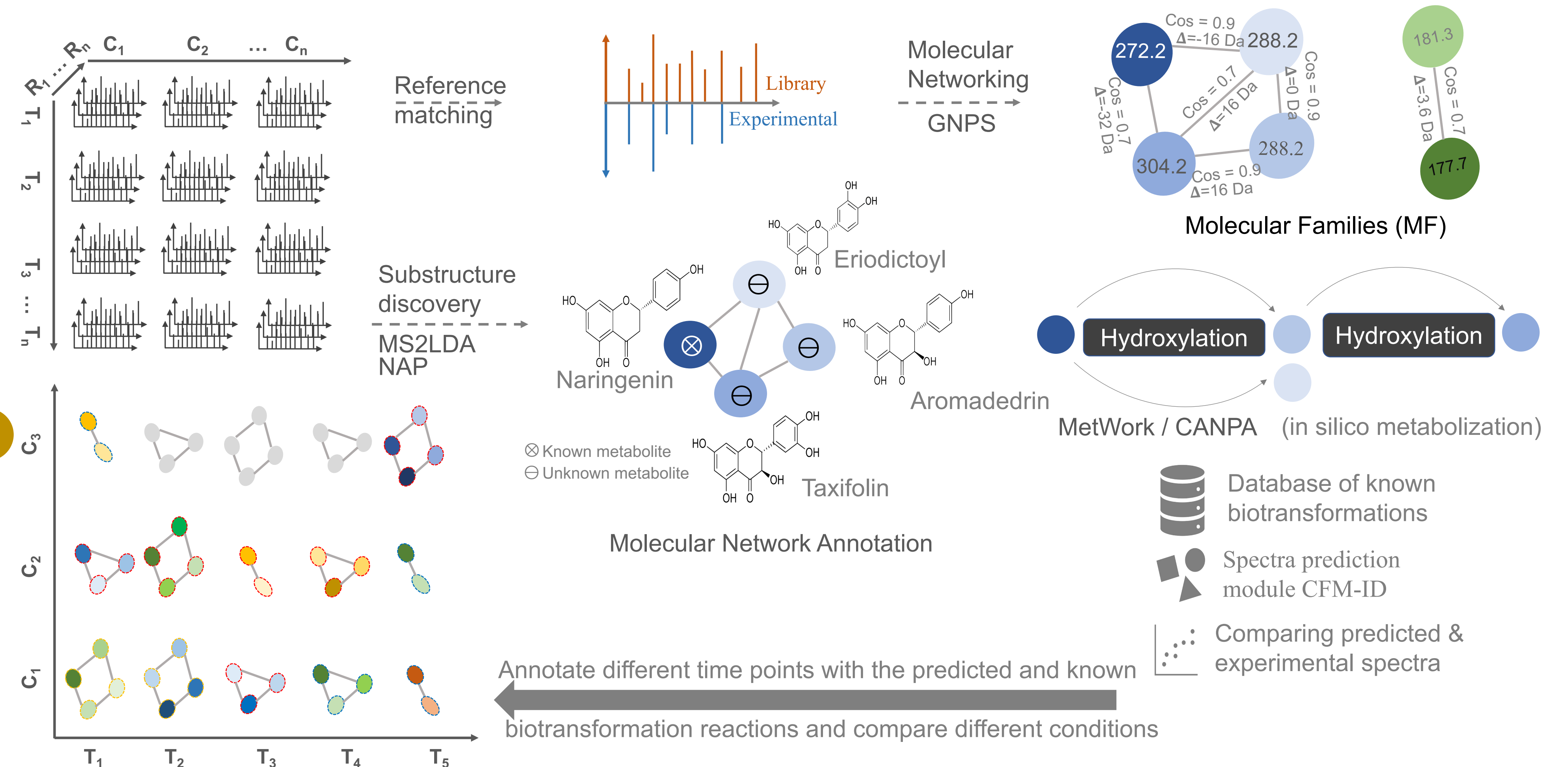
Pathway predictions

Coexpression networks

Reconstruction of coexpression modules using time-based transcriptomics data is essential to identify groups of genes involved in the same metabolic pathway. MEANtools identifies coexpression clusters and allows selection of genes present in modules which improves confidence in the overall biosynthetic pathway predictions.

Transcriptomics with time-points   Temporal analysis of transcriptome data   Coexpression network

Clustering of genes   TFBS enrichment

Expression pattern of a single gene   DEGs overlap between different conditions

weakly correlated gene

Time-based metabolomics

Time-based analysis has enormous potential to unveil transient metabolites both in terms of concentration and availability. Using time-series, spectral similarities can also be exploited to group several spectra together to form networks of fragmented features at individual time points. Here, tools like MetWork/CANPA (Ramos *et al.* 2019) can be useful for the discovery of unknown metabolites.

Reference matching

Library
Experimental

Molecular Networking
GNPS

Substructure discovery
MS2LDA
NAP

Eriodictoyl
Naringenin
Aromadedrin
Taxifolin

⊗ Known metabolite
⊖ Unknown metabolite

Molecular Network Annotation

Molecular Families (MF)

Hydroxylation   Hydroxylation

MetWork / CANPA   (in silico metabolization)

Database of known biotransformations

Spectra prediction module CFM-ID

Comparing predicted & experimental spectra

Annotate different time points with the predicted and known biotransformation reactions and compare different conditions

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