



Analyse et interprétation
des données en
protéomique et
métabolomique: points
communs et spécificités

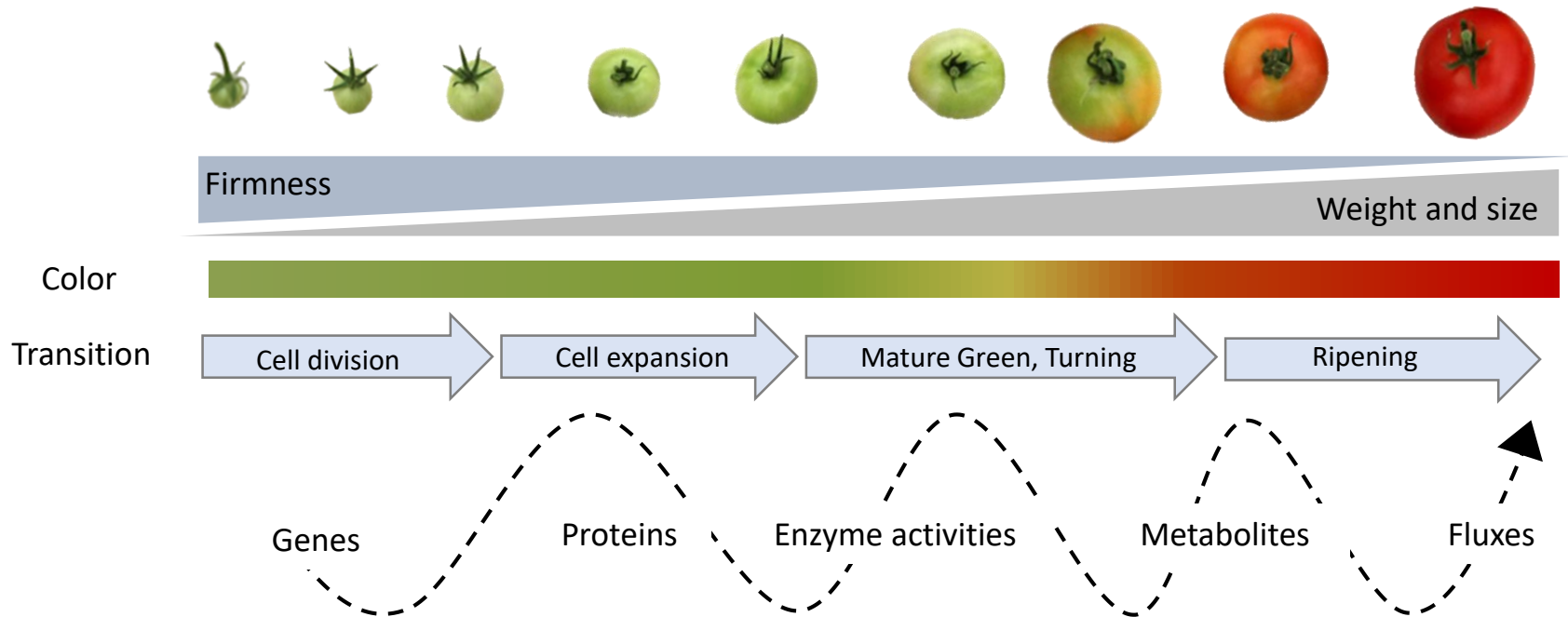
Omics integration and modeling:
Prediction of protein lifetime in tomato fruit

Belouah Isma

Tomato fruit: a model for fleshy fruit

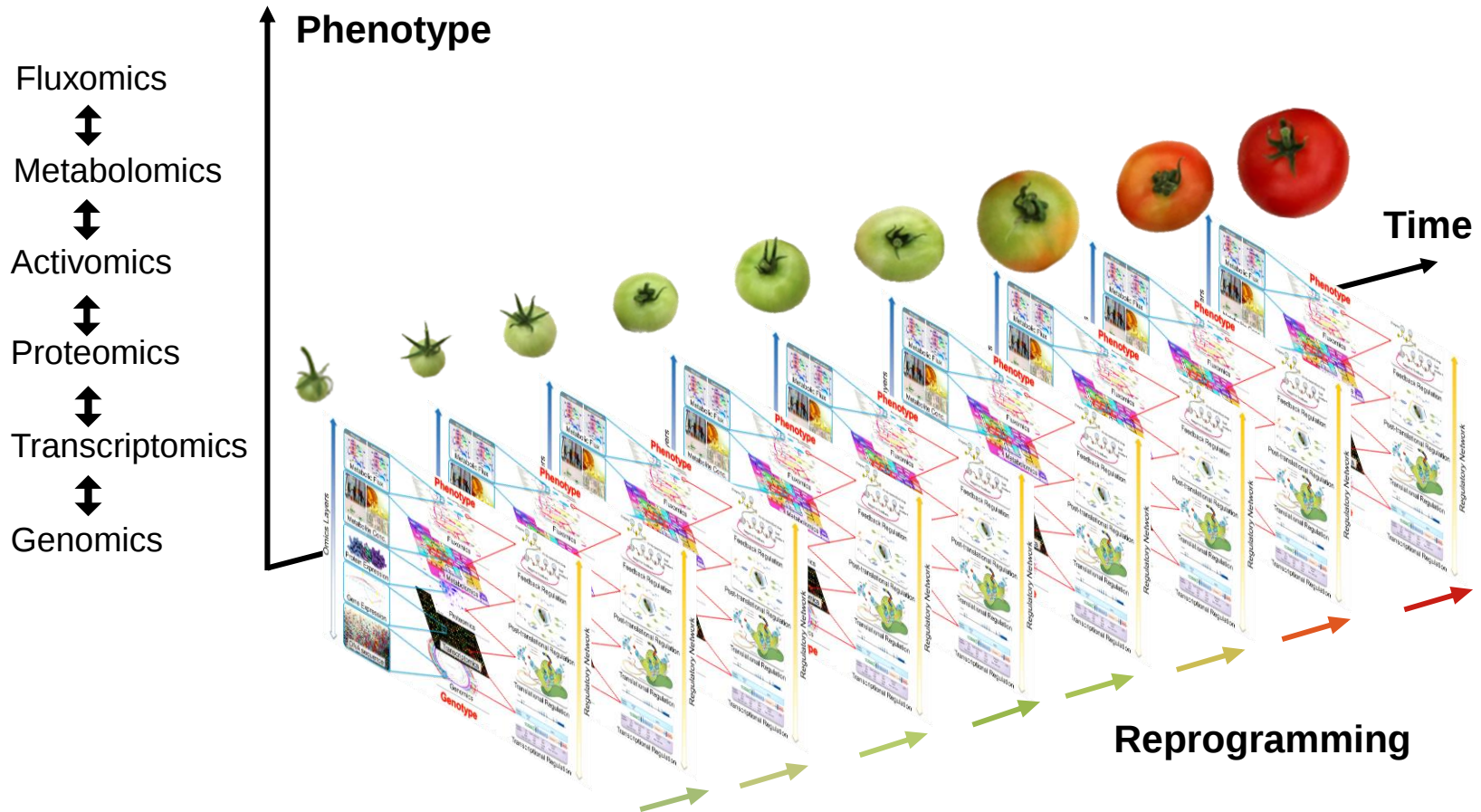
Why research on tomato fruit development:

- Agroconomical interest (human nutrition, adaptation climate changes...)
- Characterize physiological transitions
- Understand the regulation of metabolite content



Phenotype results from the interaction of Omics

- Each stage = integration of *Omics* on two dimensions (Time x *Omics*ⁿ)



Our project: 4 Omics throughout tomato fruit development

- Time-series of 9 developmental stages
- DPA = days post anthesis (fecundation)

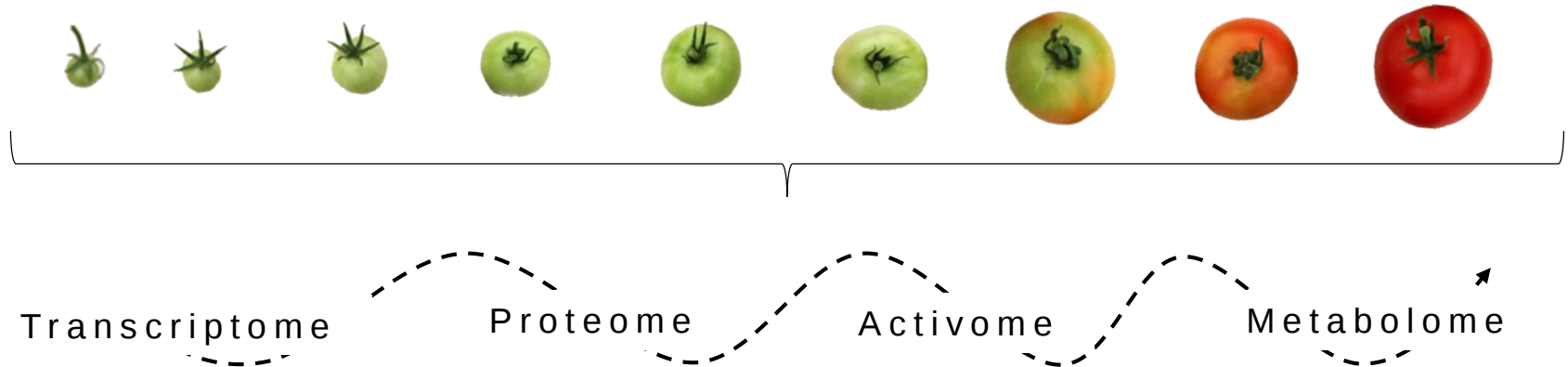


- Omics analysis performed on pericarp tissue



Pericarp tissue

Our project: 4 Omics throughout tomato fruit development



Methods:

- RNASeq
- 8 spikes injected
- fmol.gFW^{-1} :
- Label free LC-MS/MS
- XIC signal
- fmol.gFW^{-1} :
- Microplate
- Absorbance
- $\text{nmol.min}^{-1}.\text{gFW}^{-1}$:
- LC-MS, RMN
- $\mu\text{mol.gFW}^{-1}$:



HiTMe

MAHD

Our project: 4 Omics throughout tomato fruit development



Transcriptome

Proteome

Activome

Metabolome

Methods:

- RNASeq
- 8 spikes injected
- fmol.gFW^{-1} :

- **Label free**
LC-MS/MS
- XIC signal
- fmol.gFW^{-1} :

- Microplate
- Absorbance
- $\text{nmol.min}^{-1}.\text{gFW}^{-1}$:

- LC-MS, RMN
- $\mu\text{mol.gFW}^{-1}$:

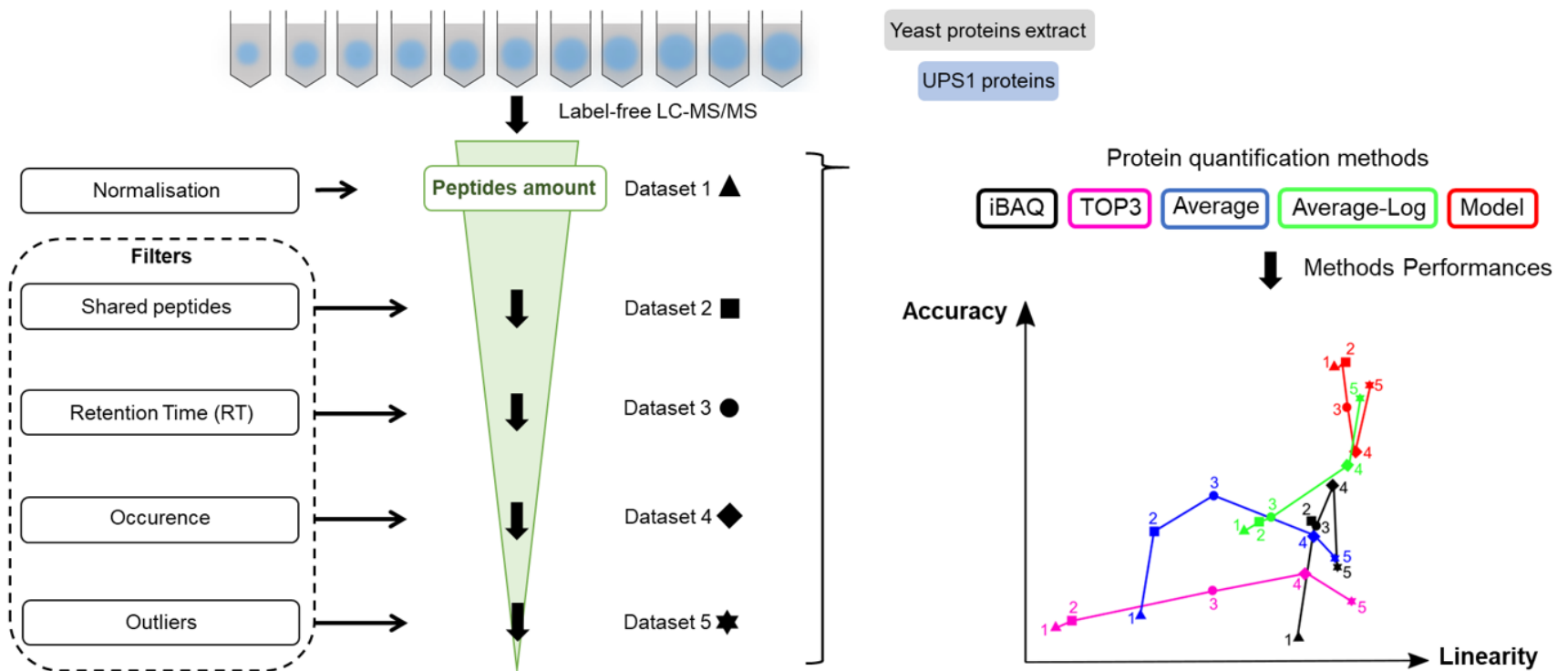


HiTMe

MAHD

Peptides intensity modeling used to quantify the abundance of proteins

Peptides intensity modeling: method of protein quantification selected among five XIC based methods.



Submitted Belouah I et al

Determination of the concentration of tomato proteins

Concentration of **2494 proteins** (fmol.gFW⁻¹) determined from their abundance

$$Protein_{i,k} = \frac{\text{abundance protein}_{i,k}}{(\sum_1^n \text{abundance protein})_k} \times (Total\ protein\ content)_k \times \frac{1}{MW_{protein_i}}$$

With,

- n the number of total proteins
- k the sample considered
- MW the molar mass (g fmol⁻¹)
- Total protein content (g gFW⁻¹)
- i the protein considered

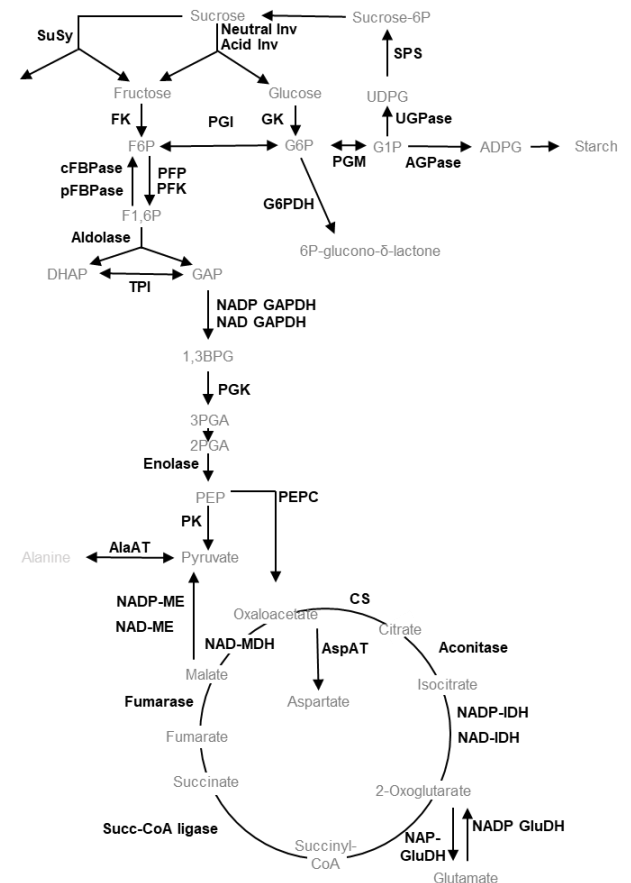
Proteome *versus* Activome

- 32 enzyme capacities expressed as enzyme concentrations

$$[\text{Enzyme}_{i,k}] = \frac{V_{max_{i,k}}}{\text{Specific activity}_{\text{Enzyme}_i}} \times \frac{1}{MW_{\text{Enzyme}_i}}$$

With,

- i the enzyme considered
- k the sample considered
- V_{max} the enzyme activity ($\text{mol} \cdot \text{min}^{-1} \cdot \text{gFW}^{-1}$)
- MW molar mass of the enzyme (g mol^{-1})
- Specific activity* ($\text{mol} \cdot \text{g}_{\text{Enzyme}}^{-1} \cdot \text{min}^{-1}$)

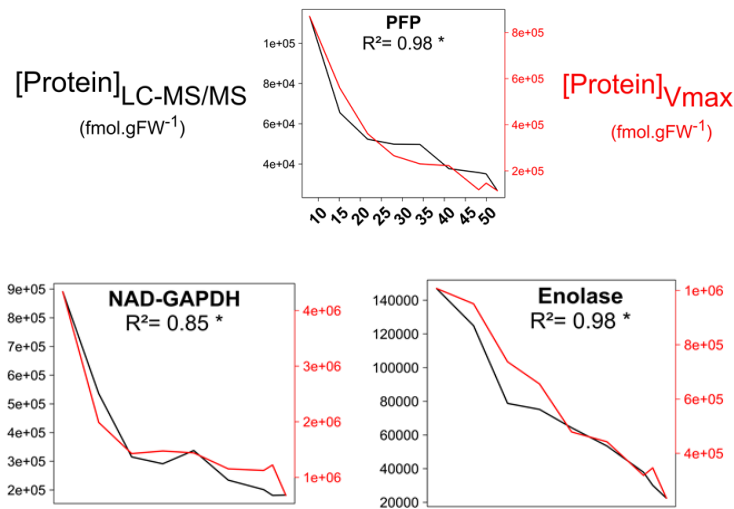


Biais et al., (2014)

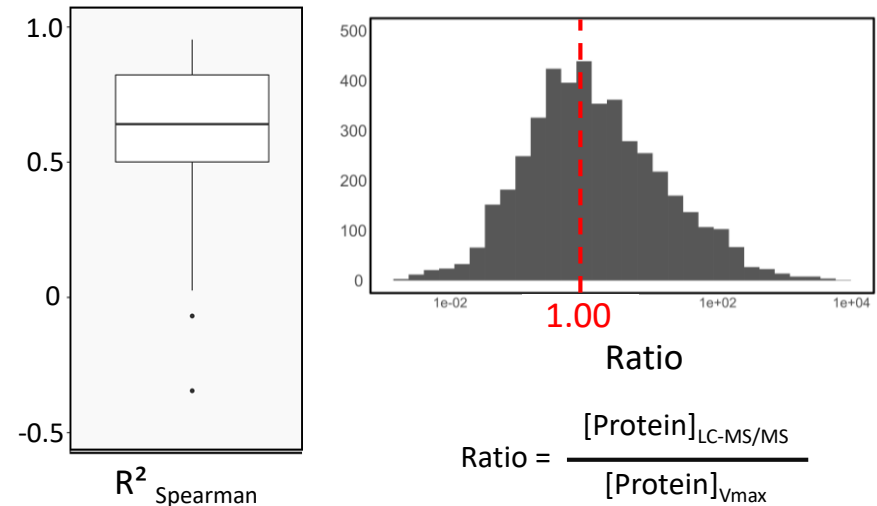
Proteome versus Activome

- 32 enzyme capacities expressed as enzyme concentrations

Three examples of correlated trends

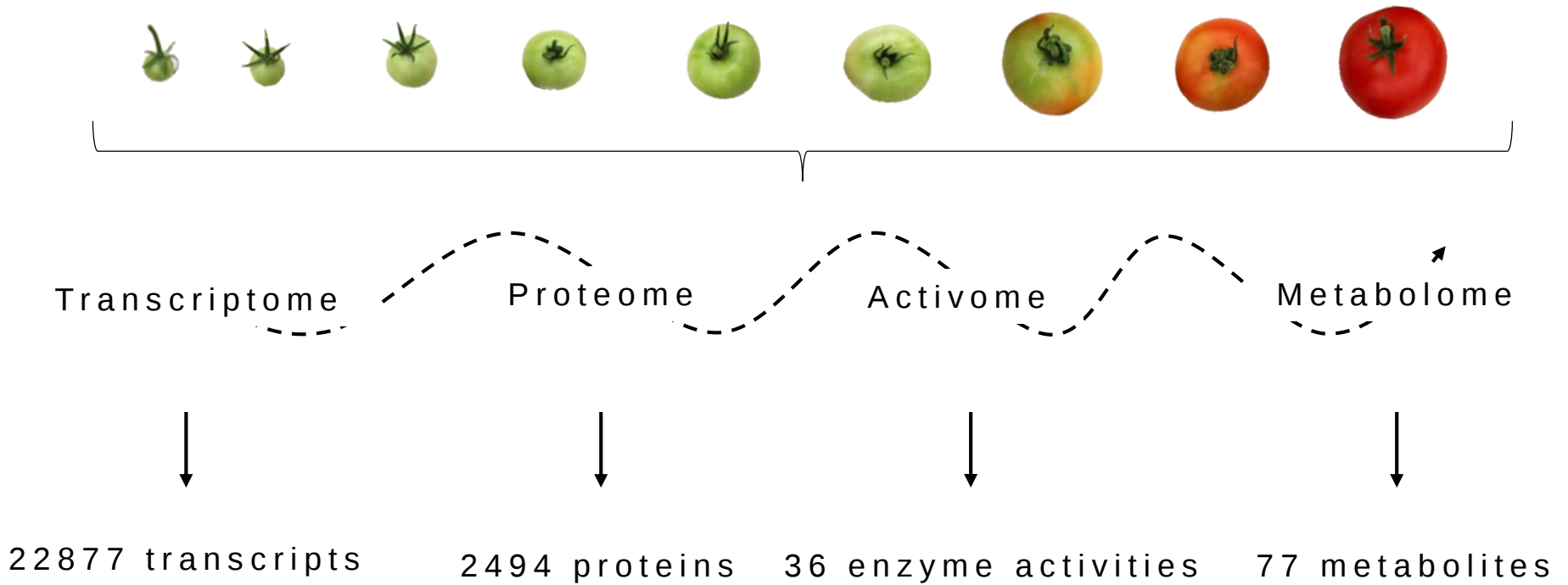


Results on the 32 enzymes



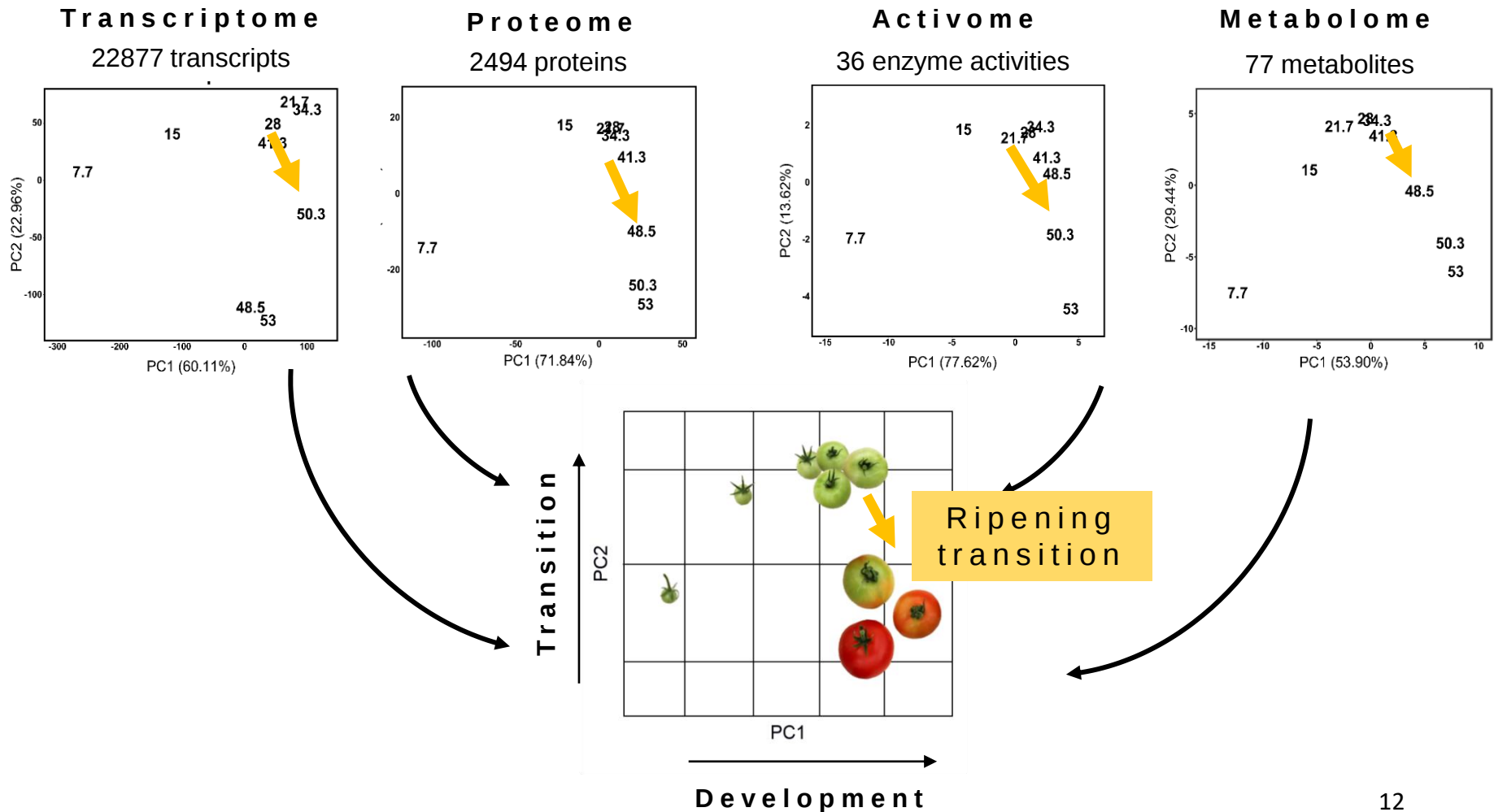
- Similar trend** of enzyme concentrations determined by both methods (*from* LC-MS/MS *vs from* Vmax)

Our project: 4 Omics throughout tomato fruit development



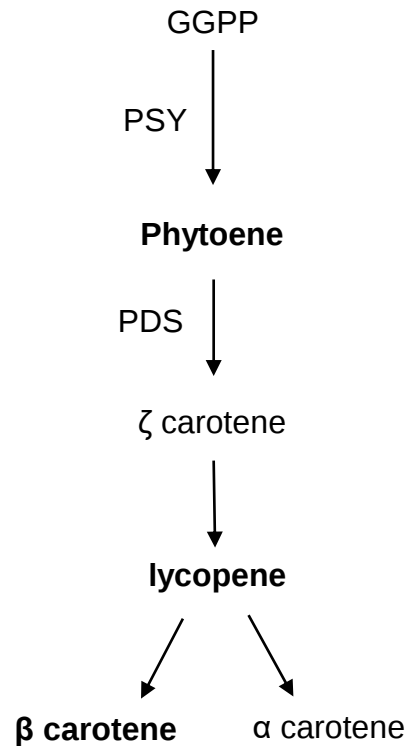
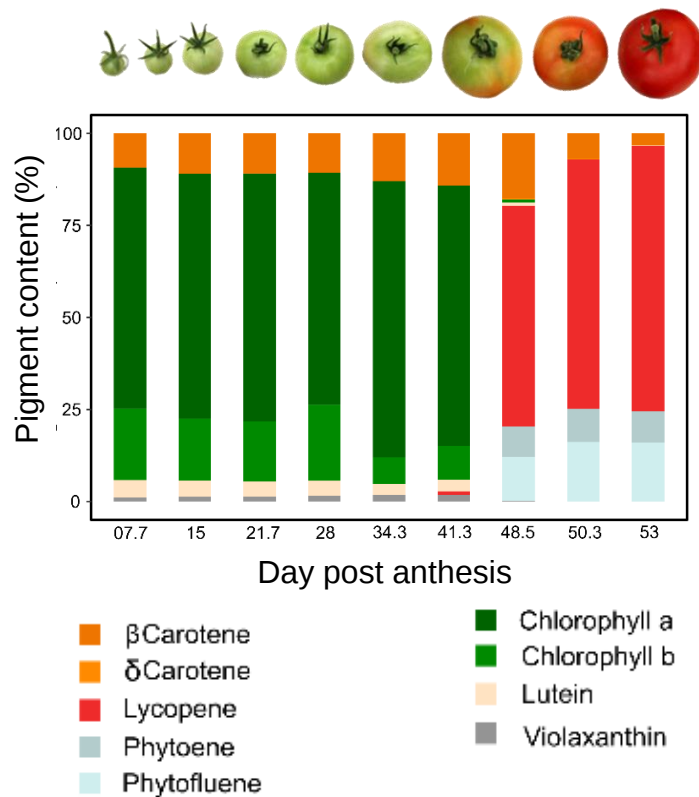
Integrate four Omics to describe transition

- Four Omics and a similar PCA profile

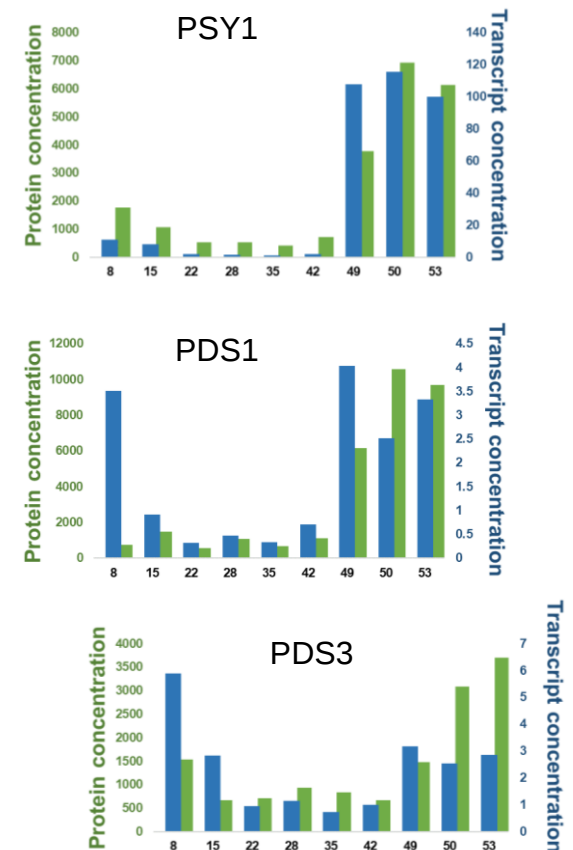


Exemple with pigments content

- Similar trend in carotenoids content and concentration of proteins and transcripts involved in pigment metabolism

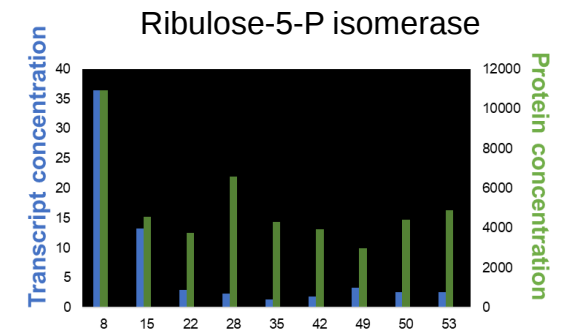
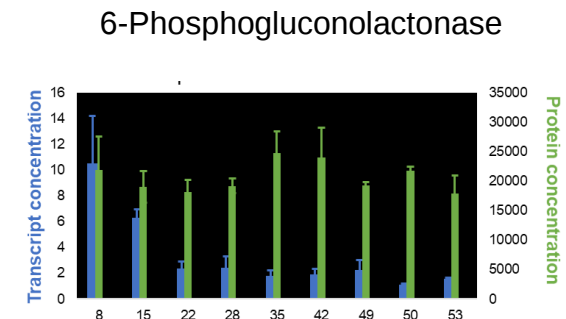
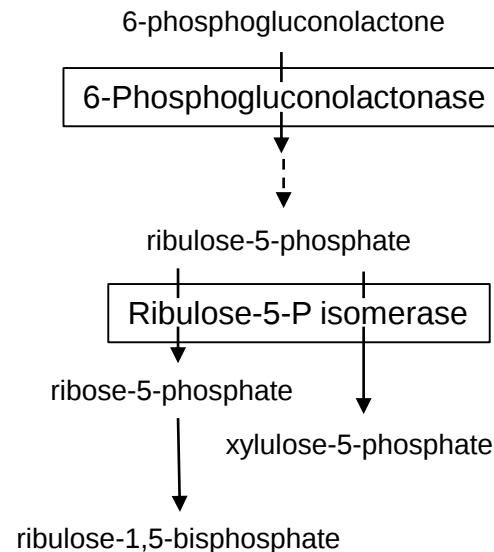
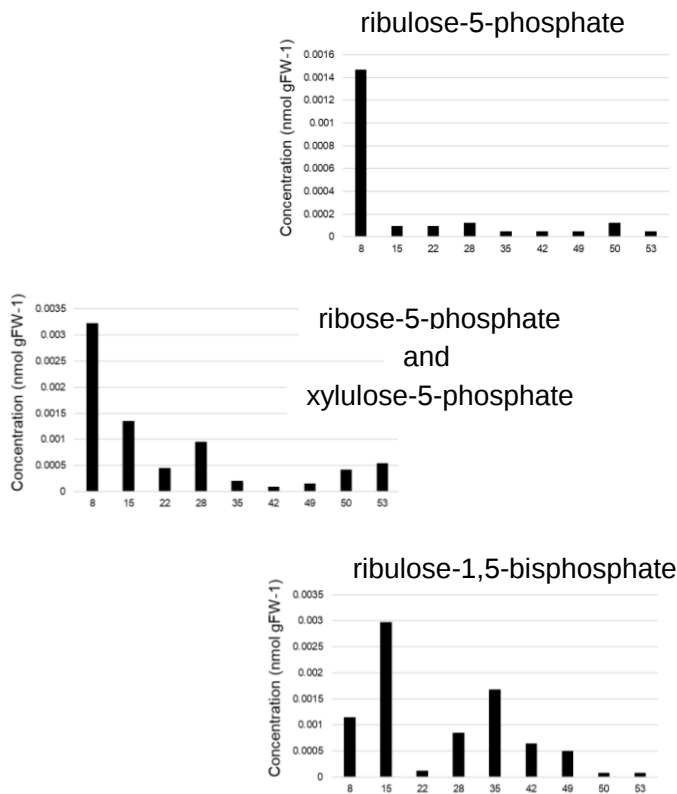


Bramley et al, (2007)



Exemple with pentose phosphate pathway

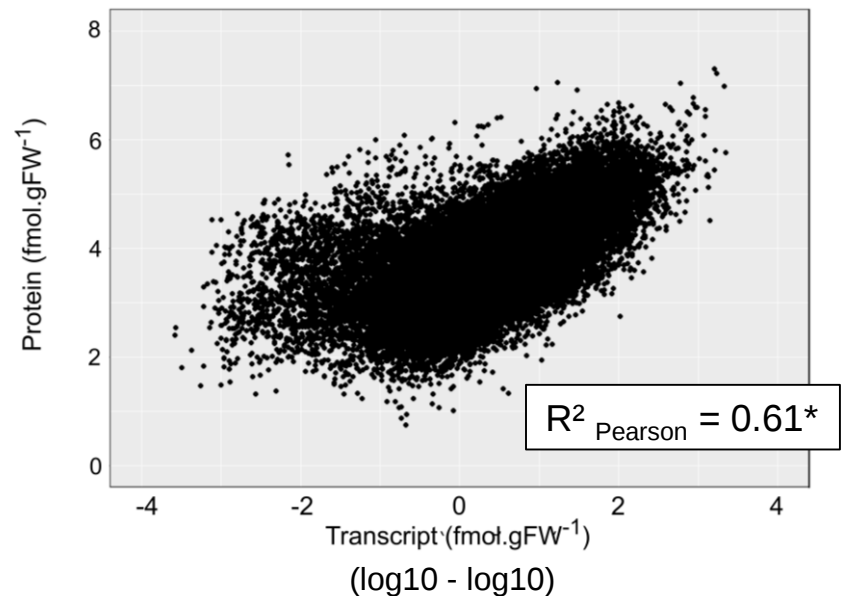
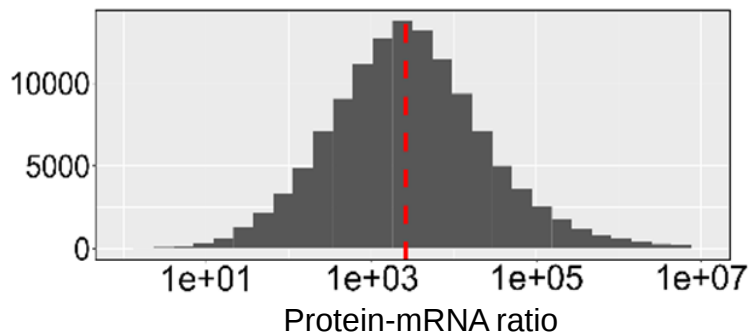
- Relationship between metabolites, proteins and transcripts is less simple



- What regulate protein content from transcript?

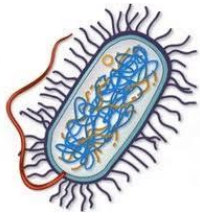
Relation between proteins and transcripts concentration

- 2490 protein-transcript pairs
- « One transcript for a thousand proteins »
- More than 60% of the protein content can be explained by the transcript level



Similar correlation in various organisms

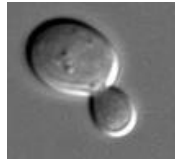
Bacteria
(E.coli)



$R^2 = 0.57$
N = 1103 pairs

Maier et al., (2009)

Yeast
(S. Cerevisiae)



$R^2 = 0.66$
N = 2044 pairs

Maier et al., (2009)

Tomato fruit
(Moneymaker)



$R^2 = 0.61$
N = 2490 pairs

Maize leaves



$R^2 < 0.58$
N = 2188 pairs

Ponnala et al., (2016)

Mammals
(Human)

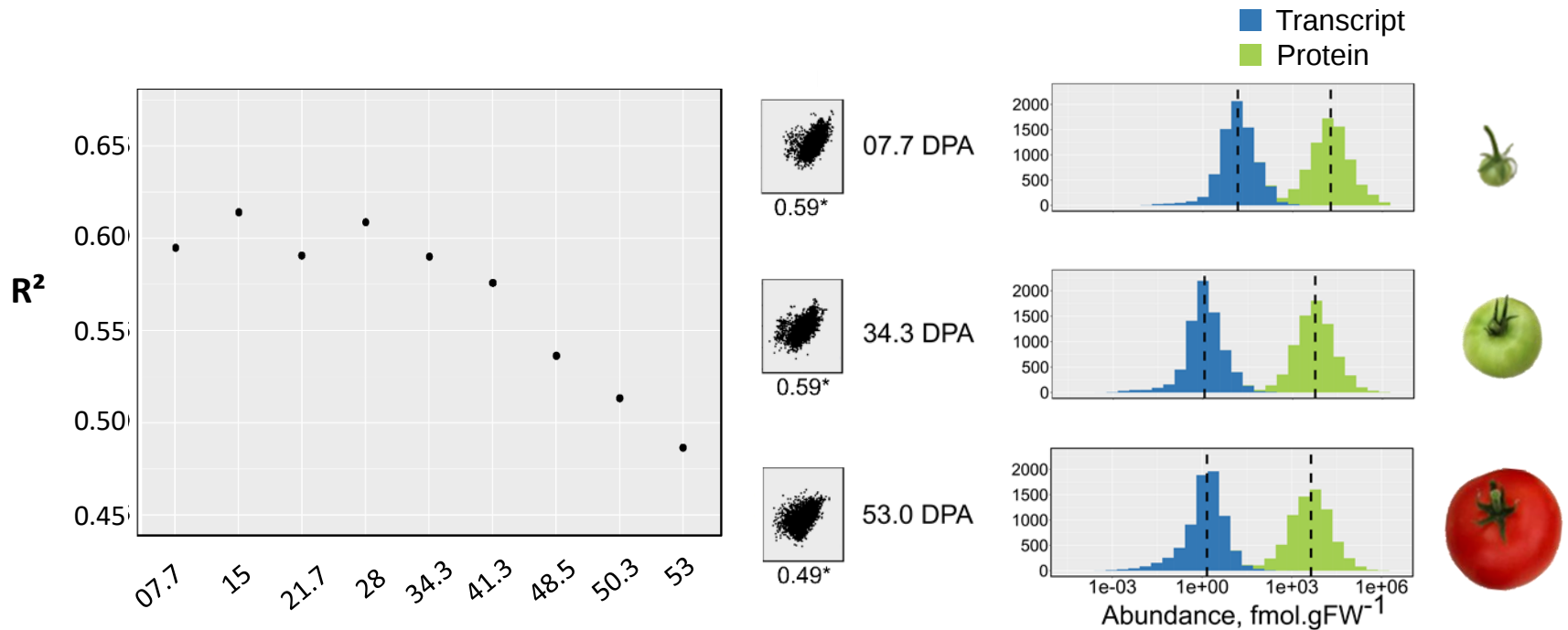


$R^2 = 0.41$
N > 5000 pairs

Schwanhäusser et al., (2011)

Relation between proteins and transcripts concentration

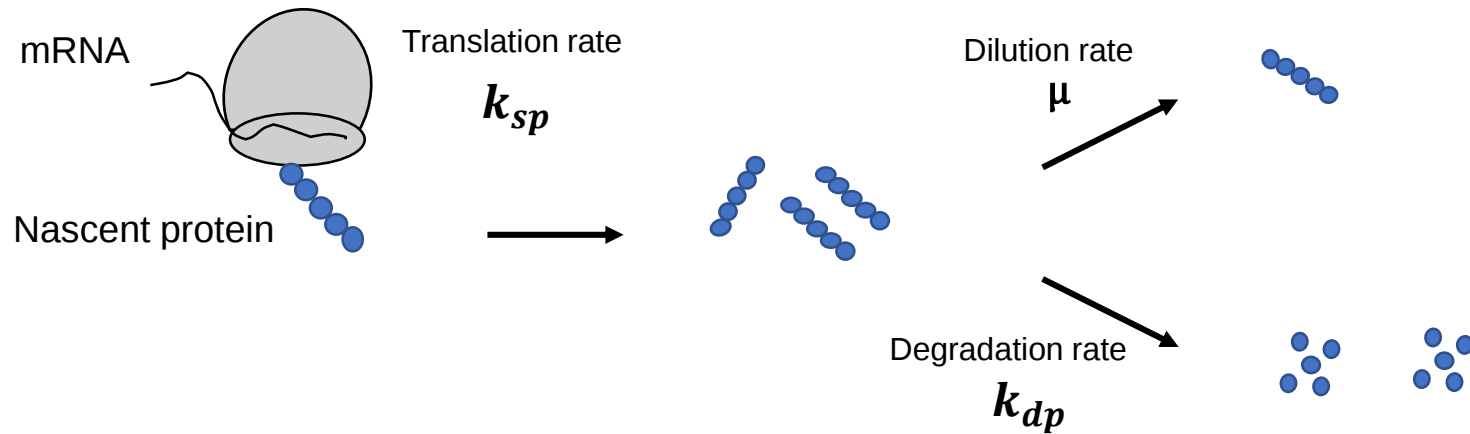
- Decrease of R^2 (Pearson) throughout tomato fruit development



- Proteins are accumulated while transcript content decreased

└─> Regulation of each protein content?

A mathematical model to predict protein lifetime



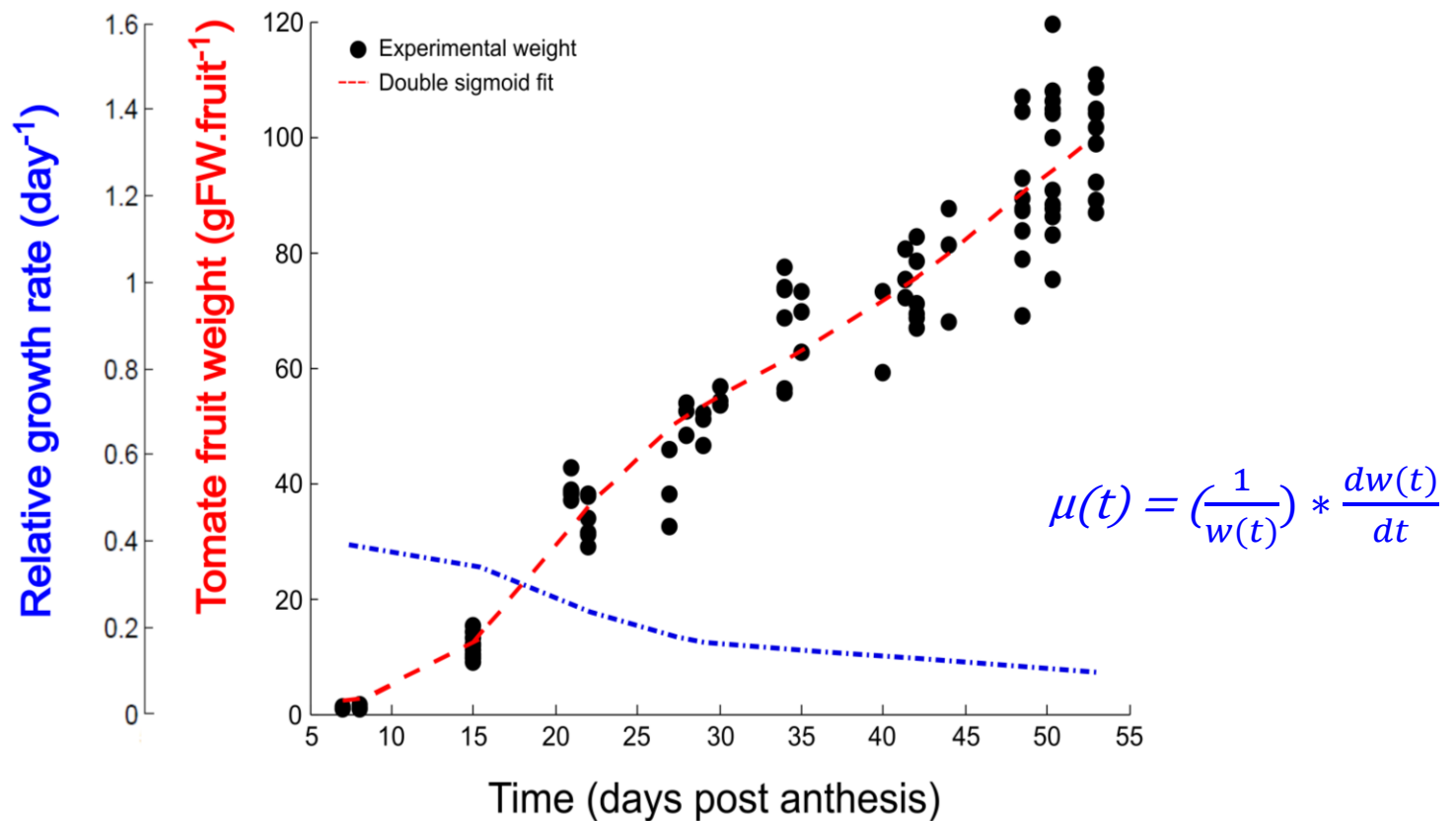
$$\frac{dp(t)}{dt} = k_{sp} r(t) - (k_{dp} + \mu(t)) p(t)$$

Dressaire et al., (2009)

With, $p(t)$ the protein concentration (fmol gFW⁻¹)
 $r(t)$ the transcript concentration (fmol gFW⁻¹)
 k_{sp} the constant synthesis rate (day⁻¹)
 k_{dp} the constant degradation rate (day⁻¹)

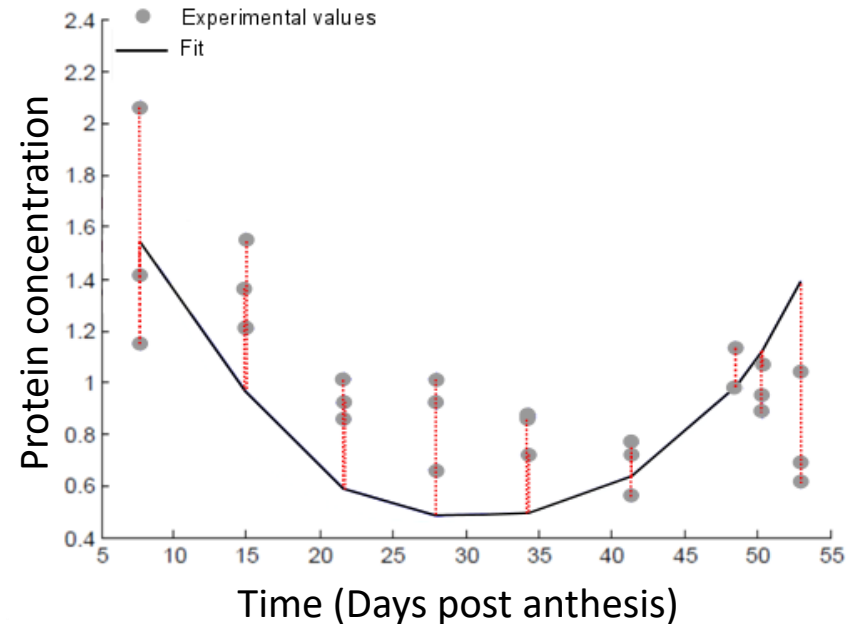
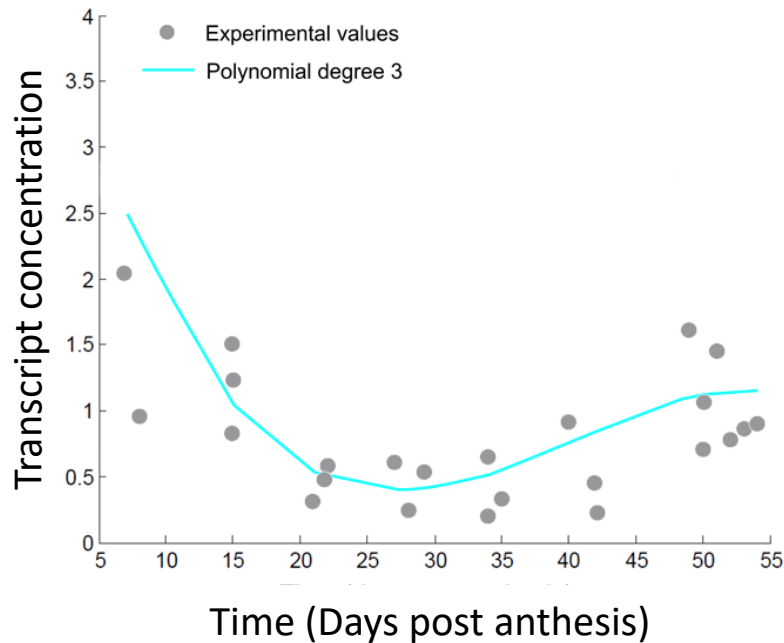
Fit of the growth rate to determine concentrations at the fruit level

$$\frac{dp(t)}{dt} = k_{sp} r(t) - (k_{dp} + \mu(t)) p(t)$$



Fit of the transcript concentration using polynomial function

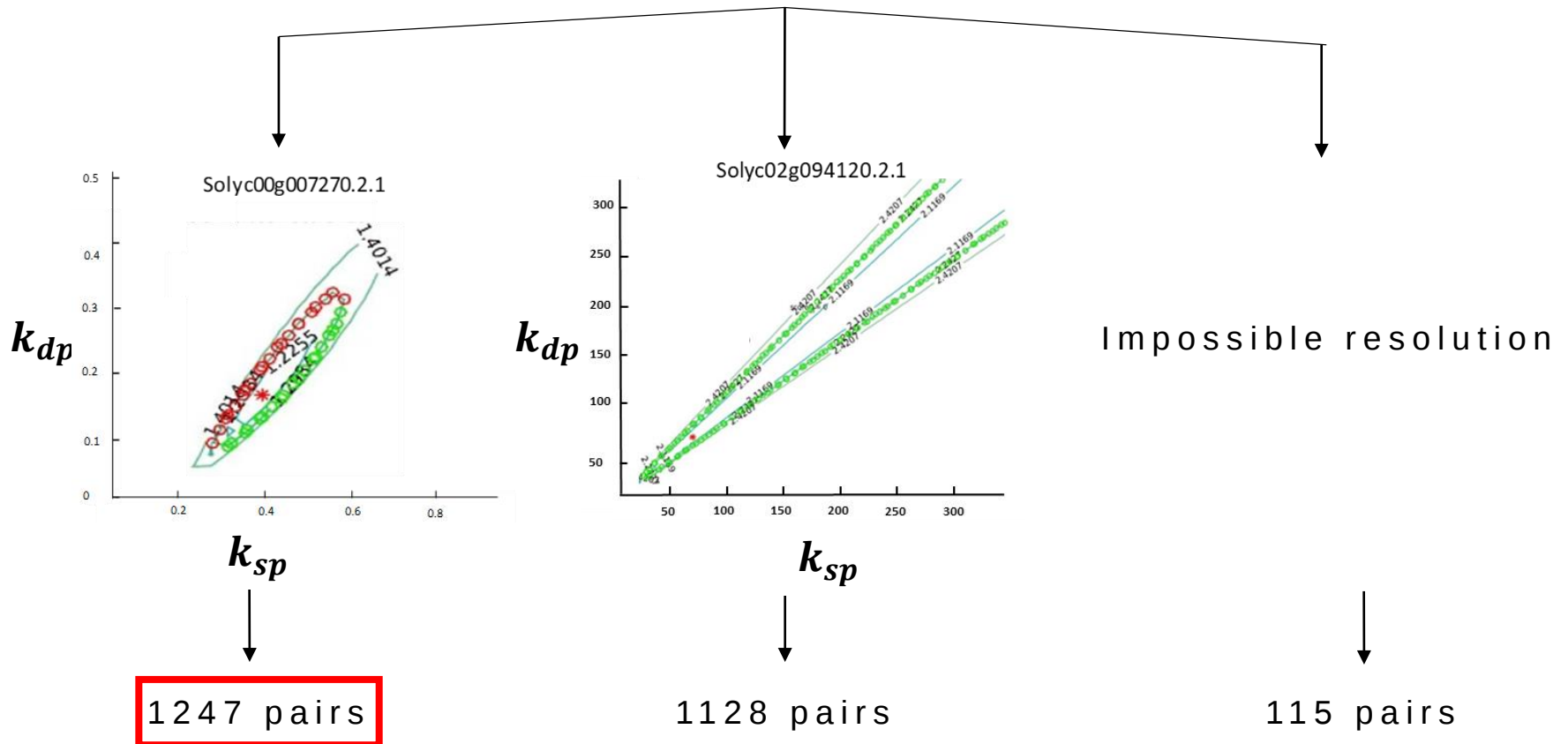
$$\frac{dp(t)}{dt} = k_{sp} r(t) - (k_{dp} + \mu(t)) p(t)$$



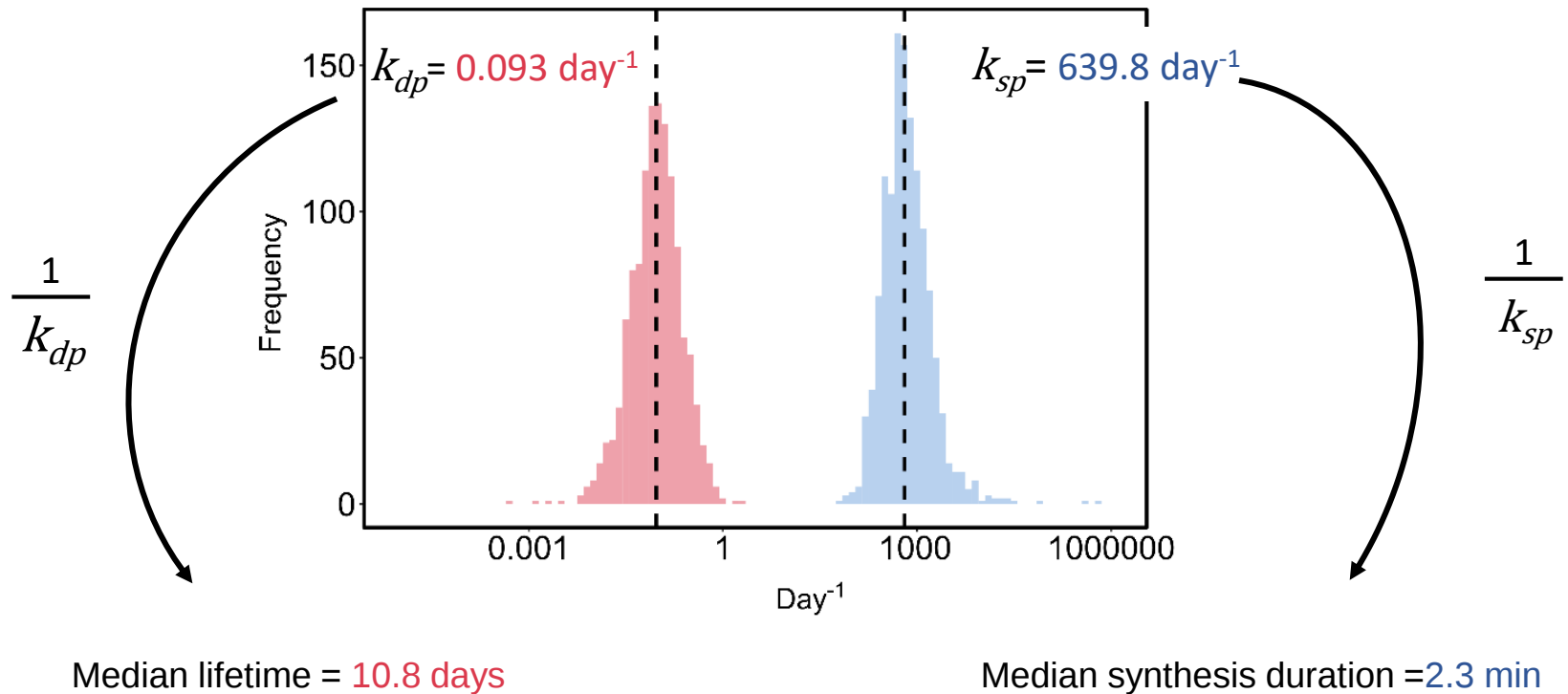
- Use of the least squares method to determine k_{sp} and k_{dp}
- Finally, 2490 synthesis and degradation rate (k_{sp} and k_{dp})

The statistical evaluation by a confidence region

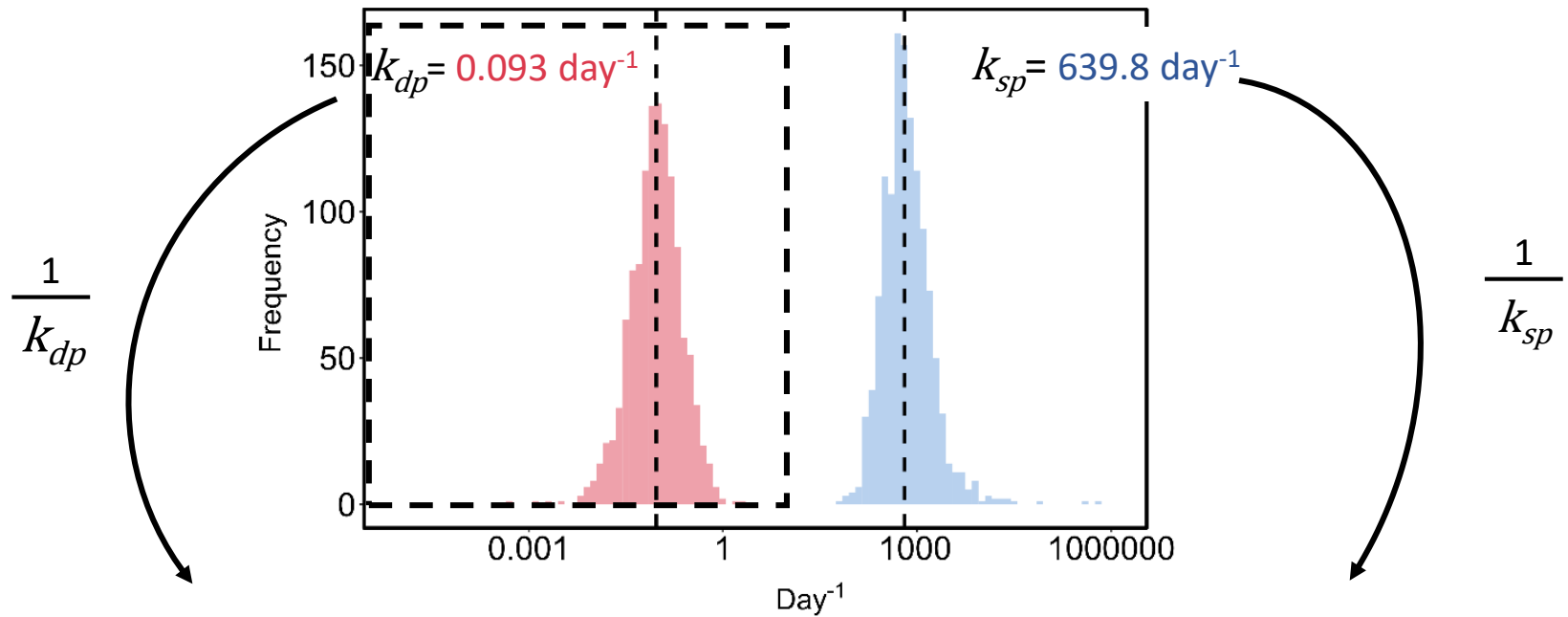
2490 protein-transcript pairs



Tomato protein lifetime 7000 higher than synthesis duration



Tomato protein lifetime 7000 higher than synthesis duration



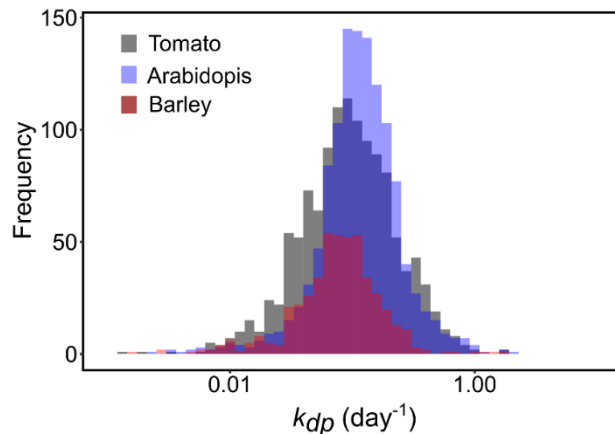
Median lifetime = 10.8 days

Median synthesis duration = 2.3 min

Comparison with k_{dp} from literature

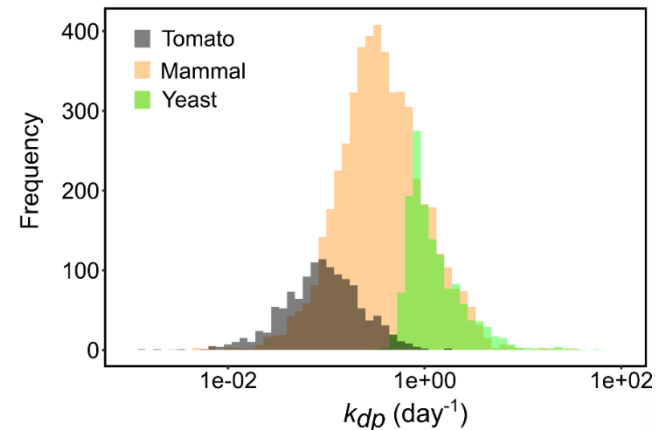
Comparison with other organisms and quantification methods

- Between plants



<u>Protein lifetime</u>		<u>Methods</u>
	10.8 days	Label-free
	8.8 days	^{15}N labeling
	13.2 days	^{15}N labeling

- Between organisms



<u>Protein lifetime</u>		<u>Methods</u>
	10.8 days	Label-free
	2.9 days	SILAC
	1 day	SILAC

- Similar k_d medians obtained with and without labeling

Lei et al (2017); Nelson et al (2014)

- Plant proteins seem to have higher lifetime than mammal and yeast proteins

Schwanhäusser et al (2010); Lahtvee et al (2017)

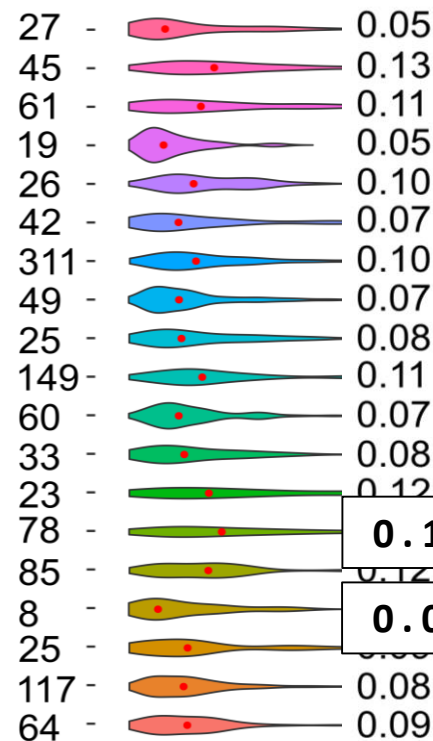
Protein lifetime and functional categories



Functional categories

Transport
Stress
Signalling
Secondary metabolism
Respiratory oxidative phosphorylation
Redox
Protein
Photosynthesis
Nucleotide metabolism
Not assigned.unknown
Miscellaneous
Lipid metabolism
Hormone metabolism
DNA, RNA binding and metabolism
Development and cellular organisation
Co-factor and vitamine metabolism
Cell wall
Carbon metabolism
Amino acid metabolism

No. k_{dp} median (day⁻¹)



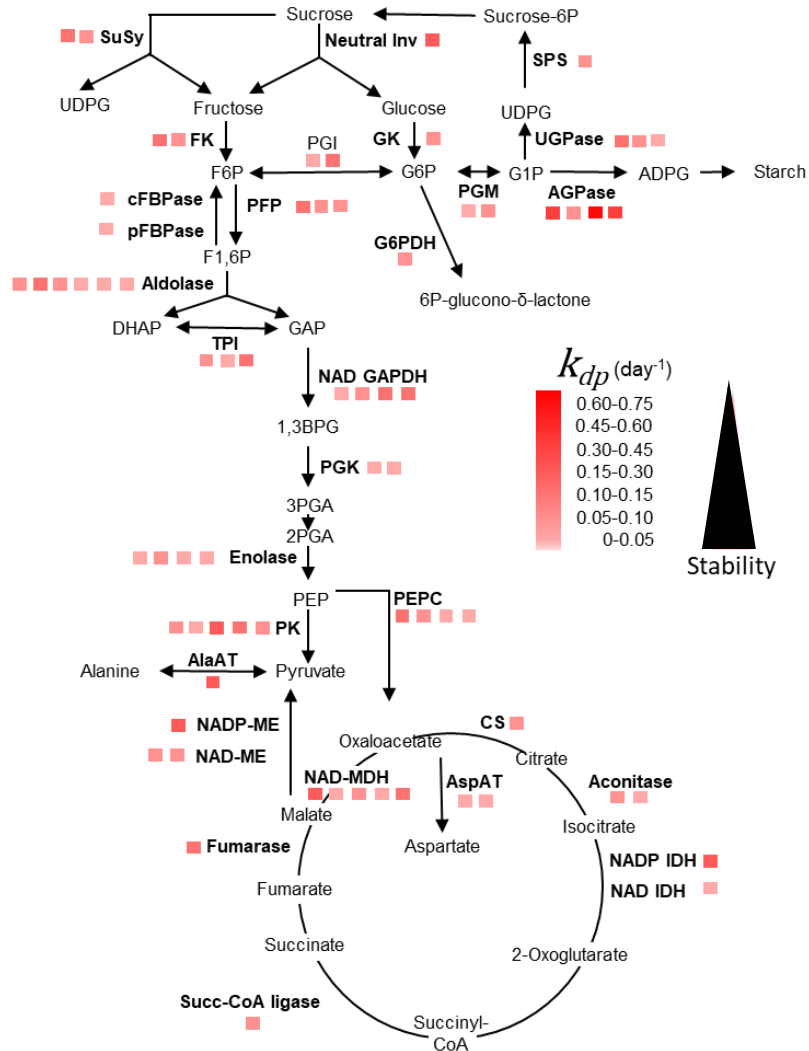
Less stable
(transcription factors?)

7.14 days

Most stable

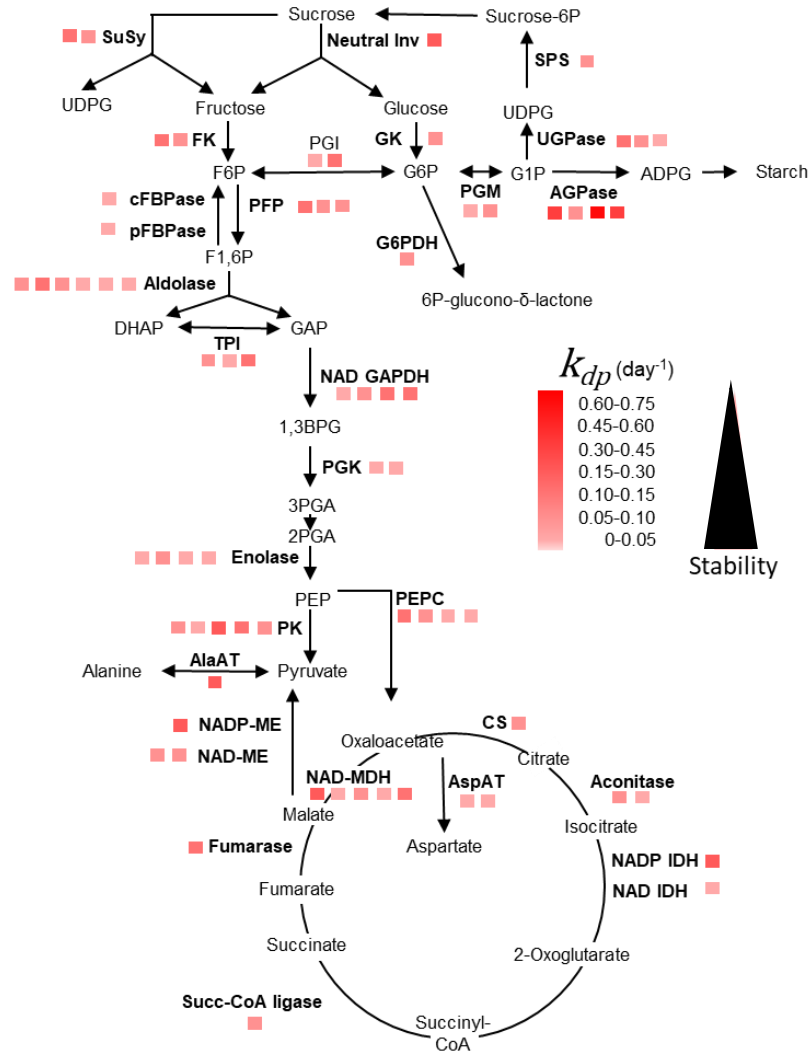
25 days

Protein lifetime and primary metabolism



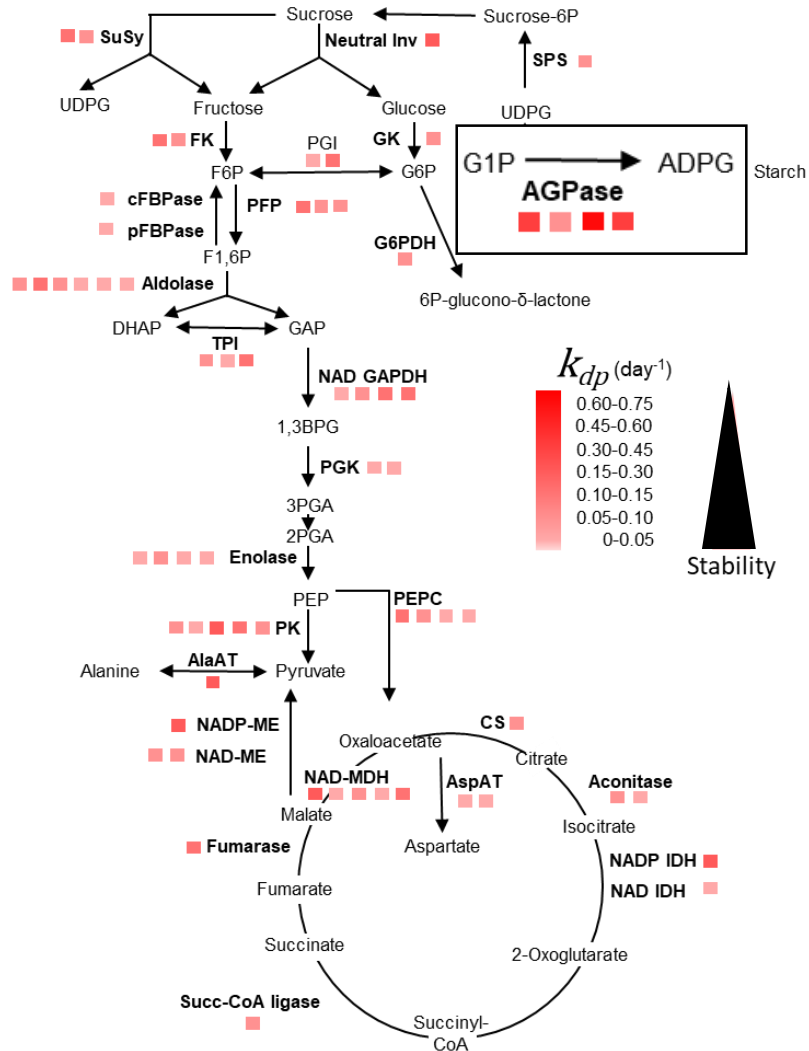
- k_{dp} range : 1.3 to 106 days

Protein lifetime and primary metabolism



- k_{dp} range : 1.3 to 106 days
- Isoform differences

Protein lifetime and primary metabolism



- k_{dp} range : 1.3 to 106 days
- Isoform differences
- AGPase: starch metabolism

Isoforms	k_{dp} (day ⁻¹)	Lifetime (day)
AGPase-1	0.37	2.69
AGPase-2	0.06	15.6
AGPase-3	0.79	1.27
AGPase-4	0.35	2.84

To conclude

- A unique dataset of quantitative omics throughout a time-serie
- Determination of the most adapted method of protein quantification
- Quantification of the concentration of tomato proteins
- Tomato protein timelife is 10.8 days (median)
- Tomato protein synthesis duration is 2.3 min (median)
- Identification of potential point of regulation (AGPase?)

Perspectives

- Relationship between degradation constants and biochemical properties (hydropathy...)
- Difference between organisms: temperature, growth rate
- Continue the integrative analysis of the four omics

Thanks for your attention

And to the contributors



Sékolène Augé
Master internship

