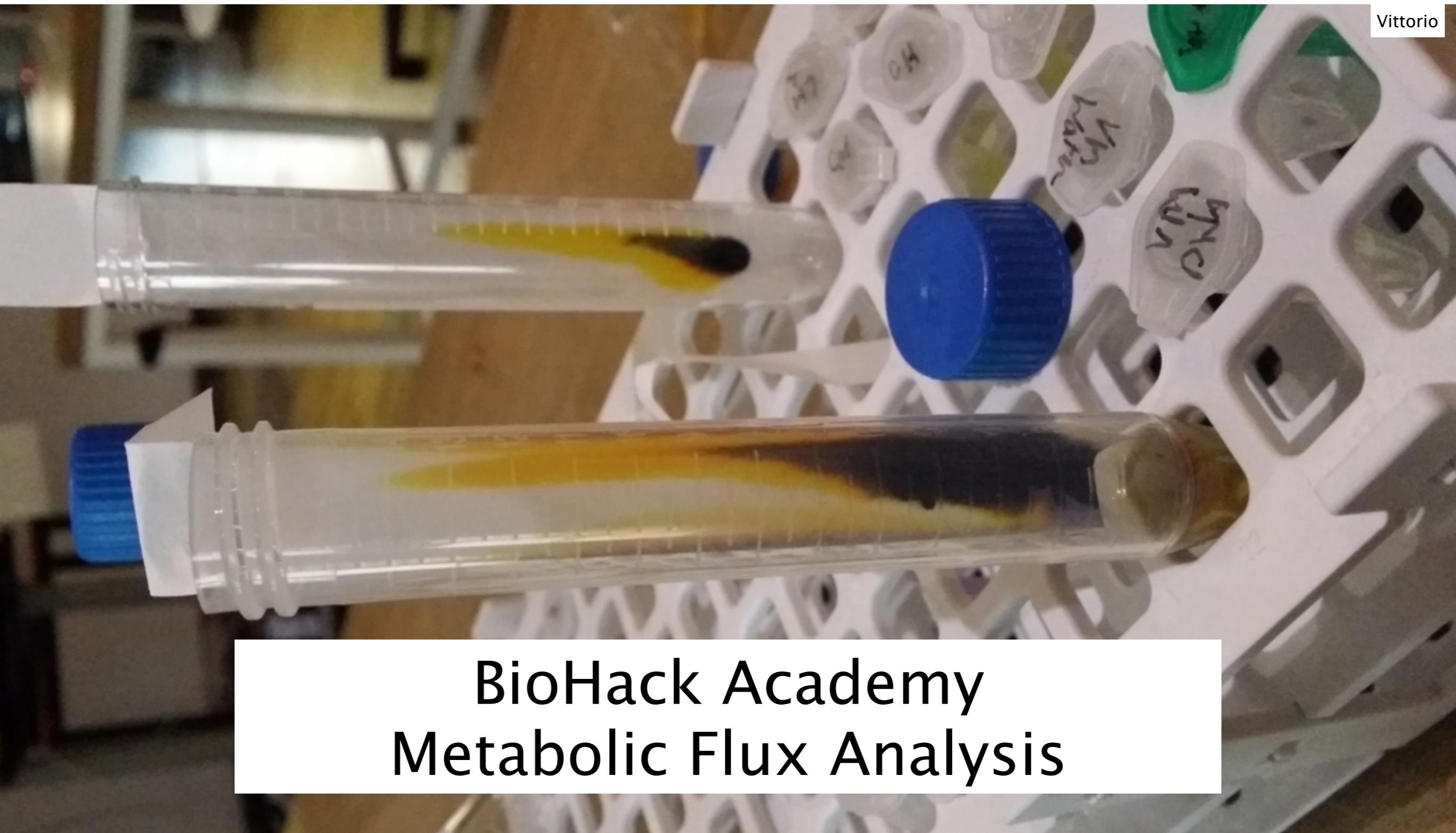




waag society

institute for art, science and technology

Vittorio

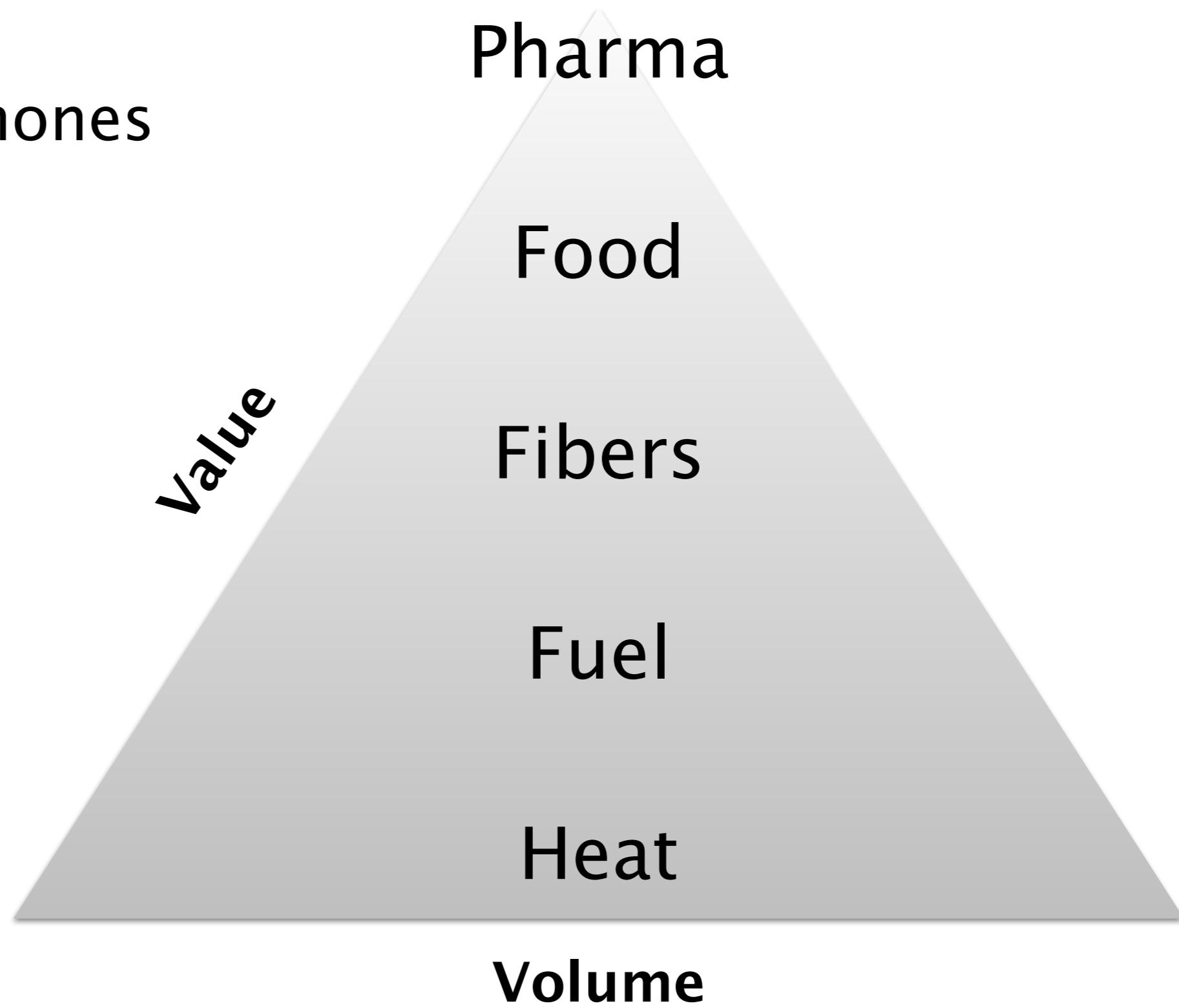


BioHack Academy
Metabolic Flux Analysis



Bioreactor value pyramid

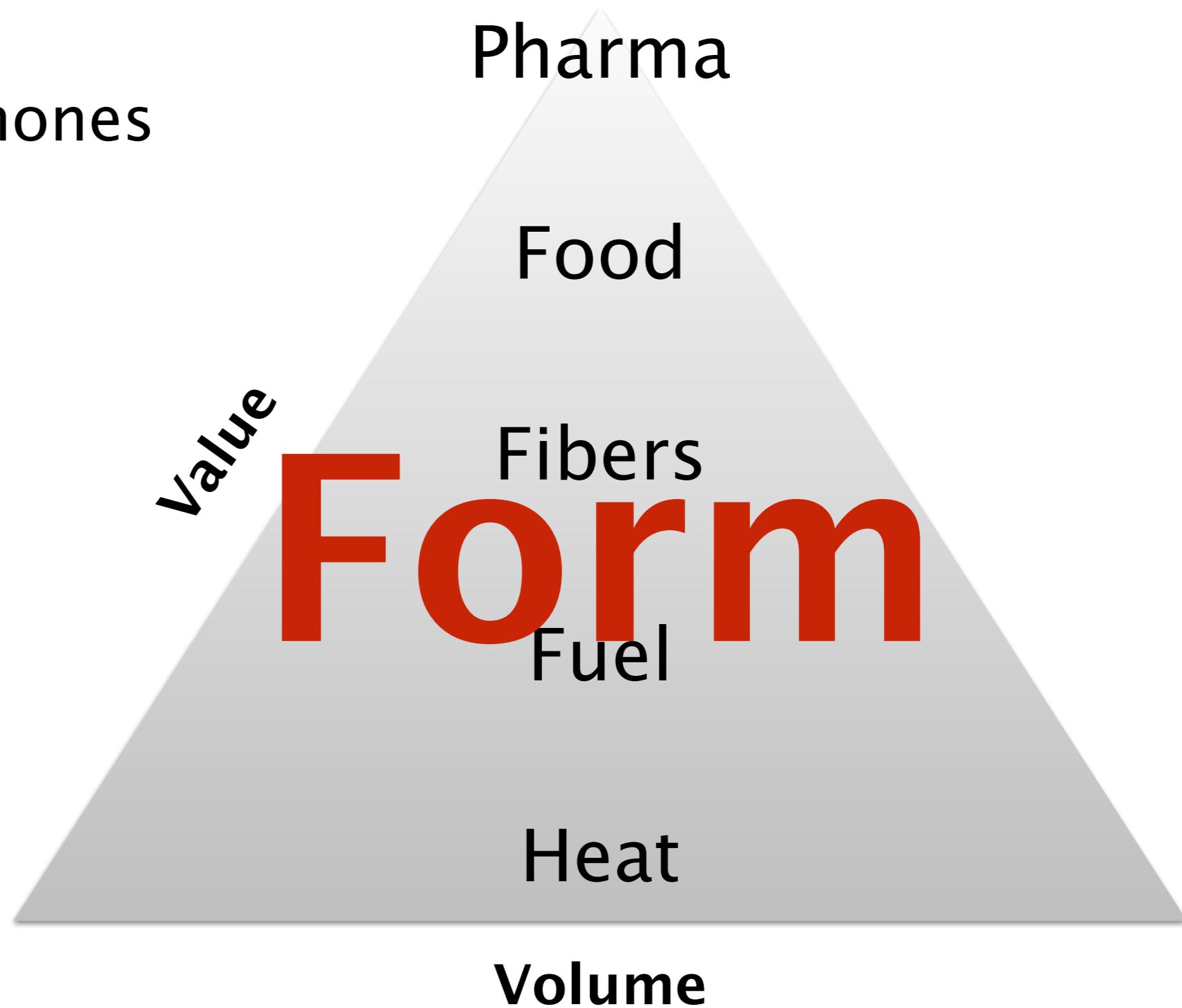
- Antibiotics
- Steroids / hormones
- Vitamins
- Proteins
- Sugars
- Acids





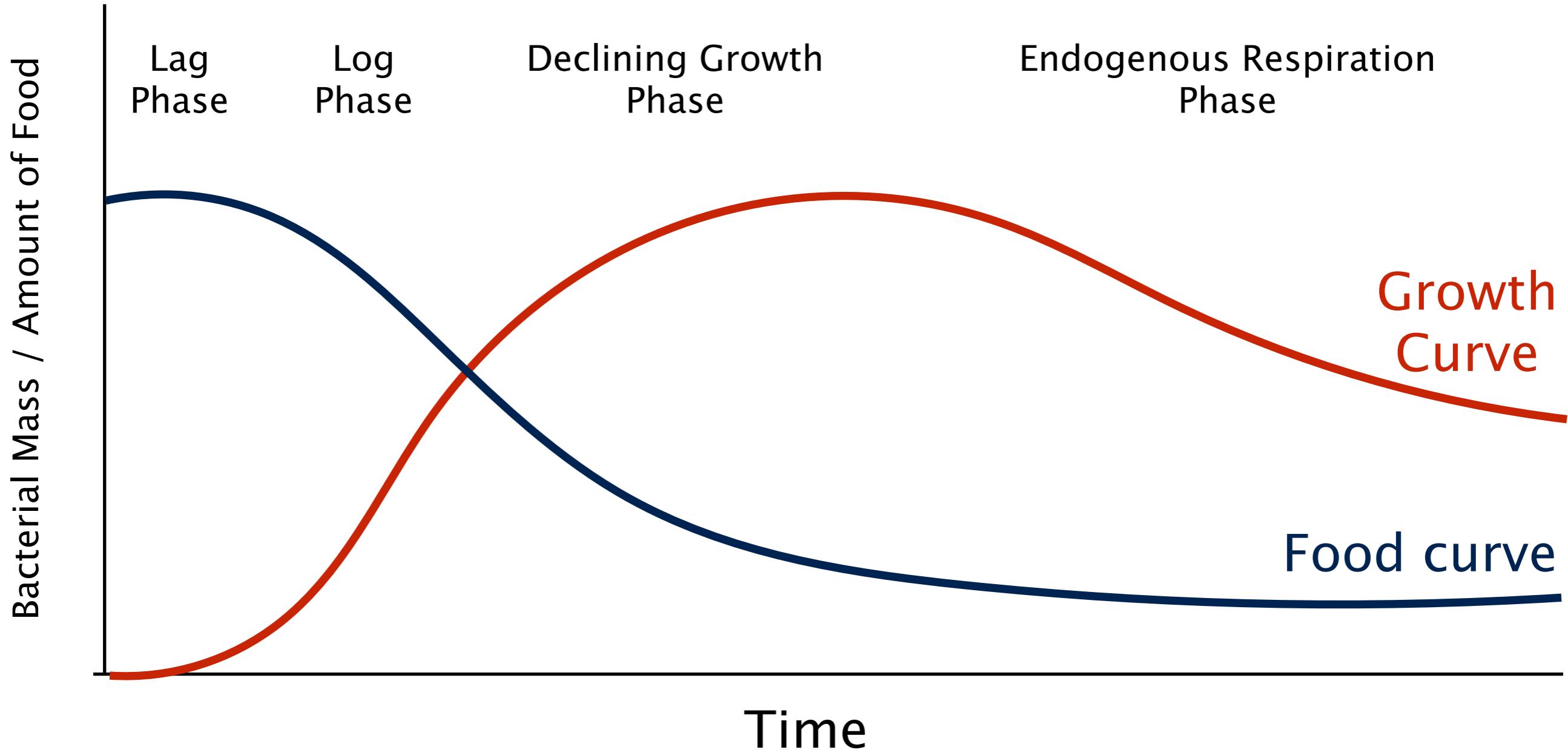
Bioreactor value pyramid

- Antibiotics
- Steroids / hormones
- Vitamins
- Proteins
- Sugars
- Acids



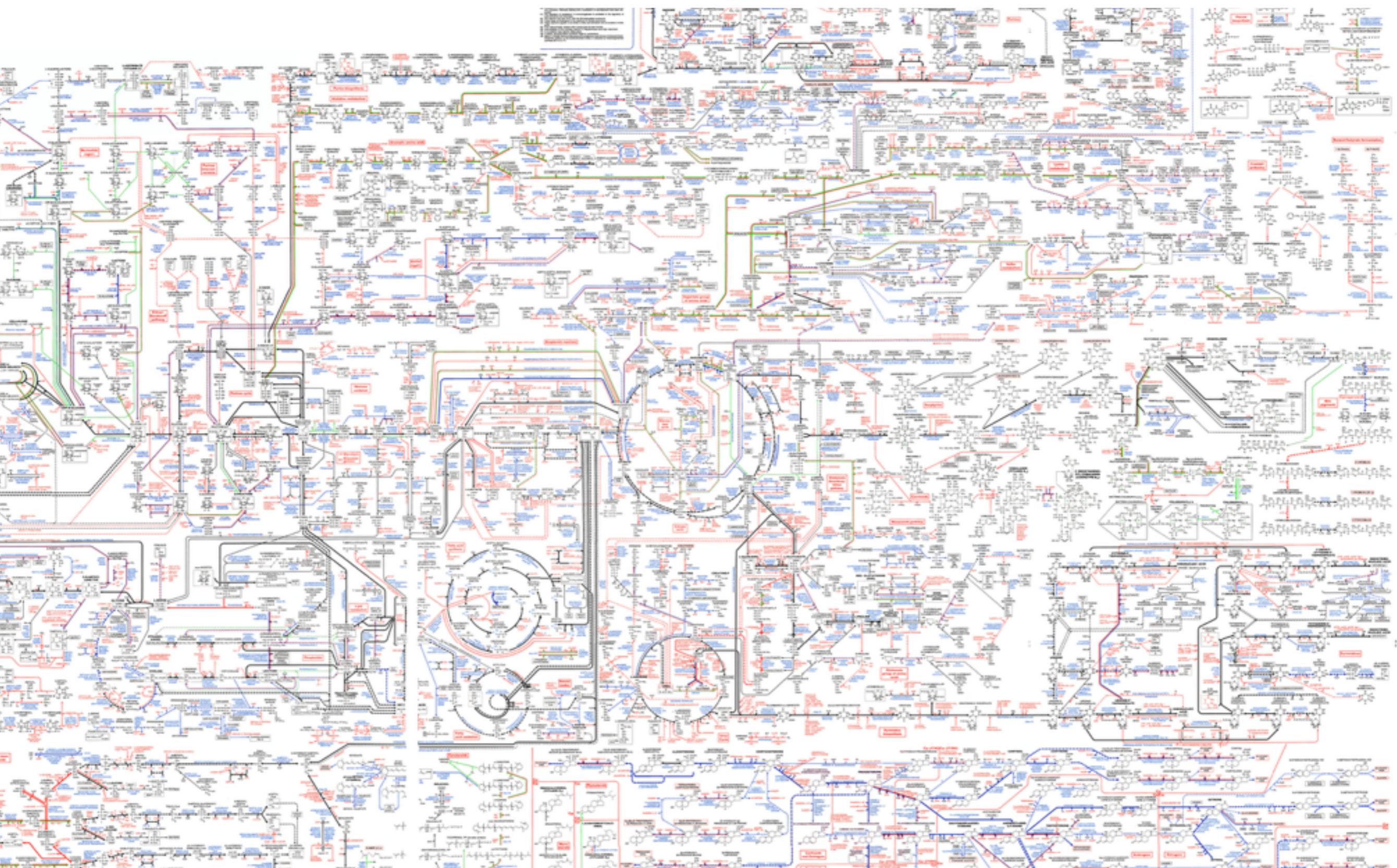


Bacterial growth curve – Batch





Metabolic Pathways





Production pathway?

Tryptophan

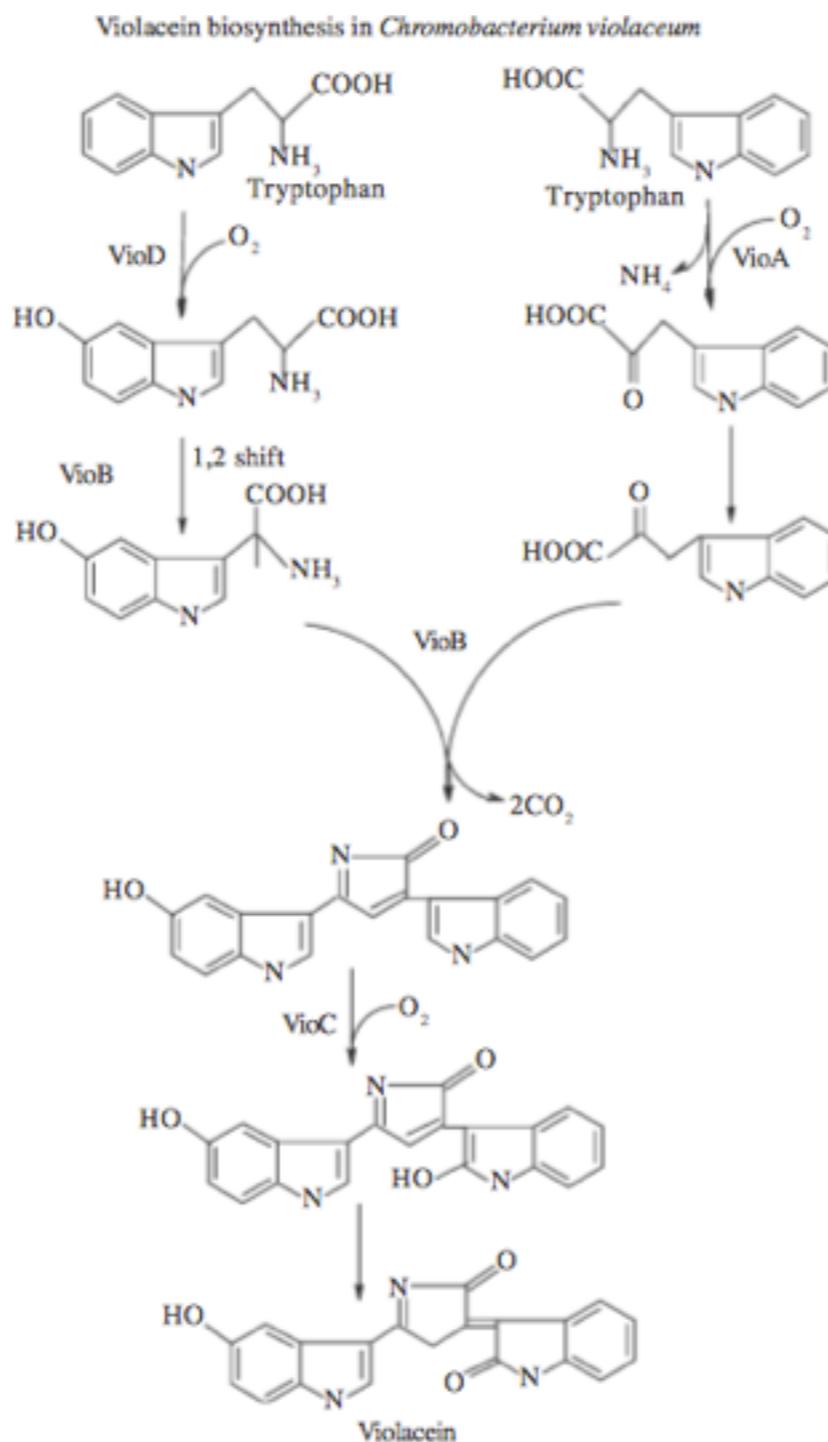


Figure 2. Violacein biosynthesis, as proposed by August et al., 2000. VioA, VioB, VioC, and VioD are the gene products of the biosynthesis operon, encoding nucleotide-dependent monooxygenases and a protein similar to a polyketide synthase (VioB).



J. Lividum canvas

BioFactory
canvas



330 mL



Nutrient
Broth
Glycerol
Tryptoph.
C
N
P
O₂
S



48h



No



volume



time



light

Yes

O₂

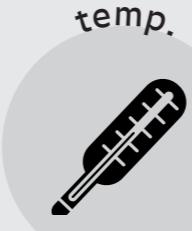


stirrer



mass

15



temp.



absorbance

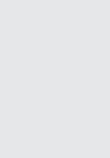


pH

1 mL?



oxygen



observations

day #

day #

day #

day #

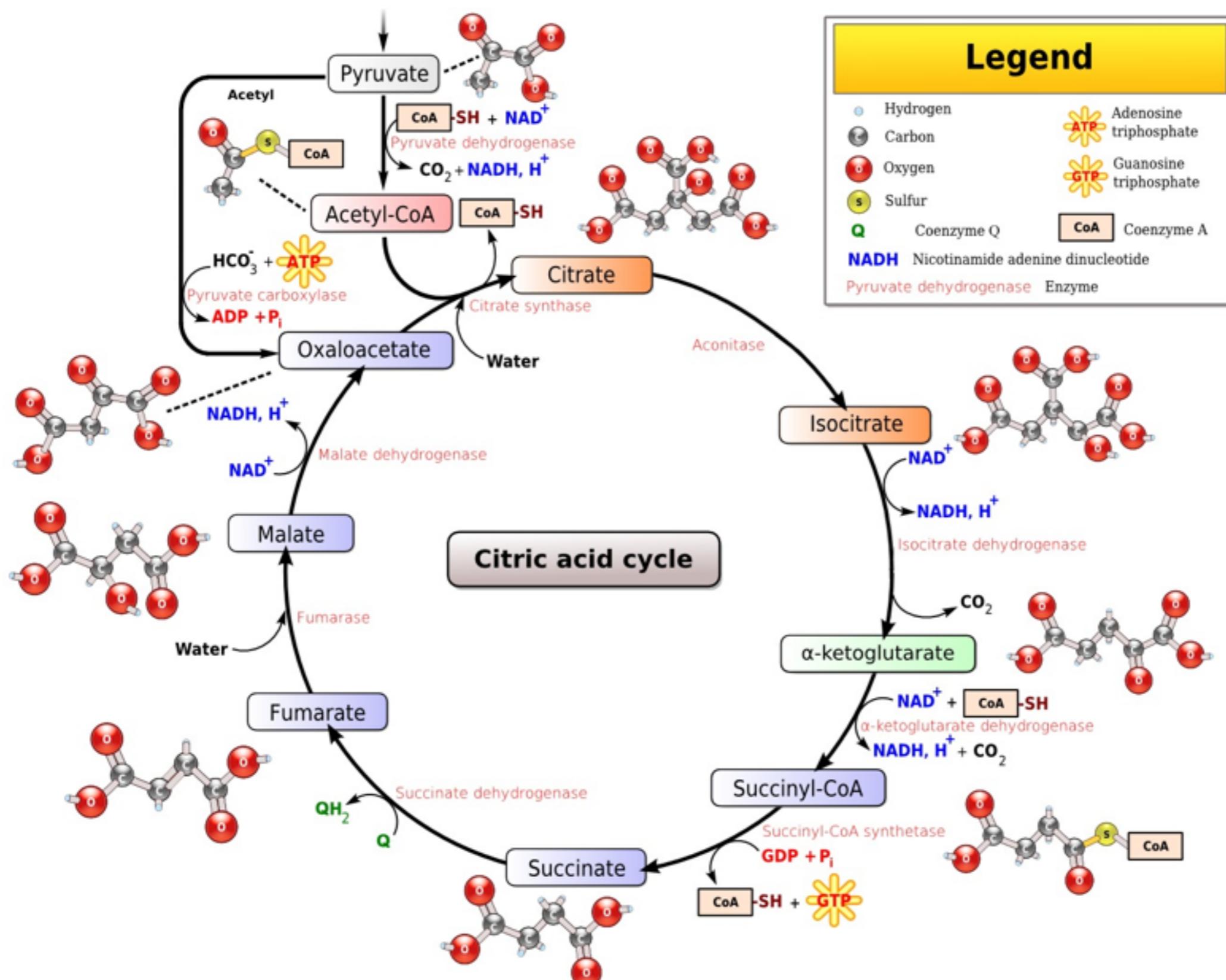
day #



material

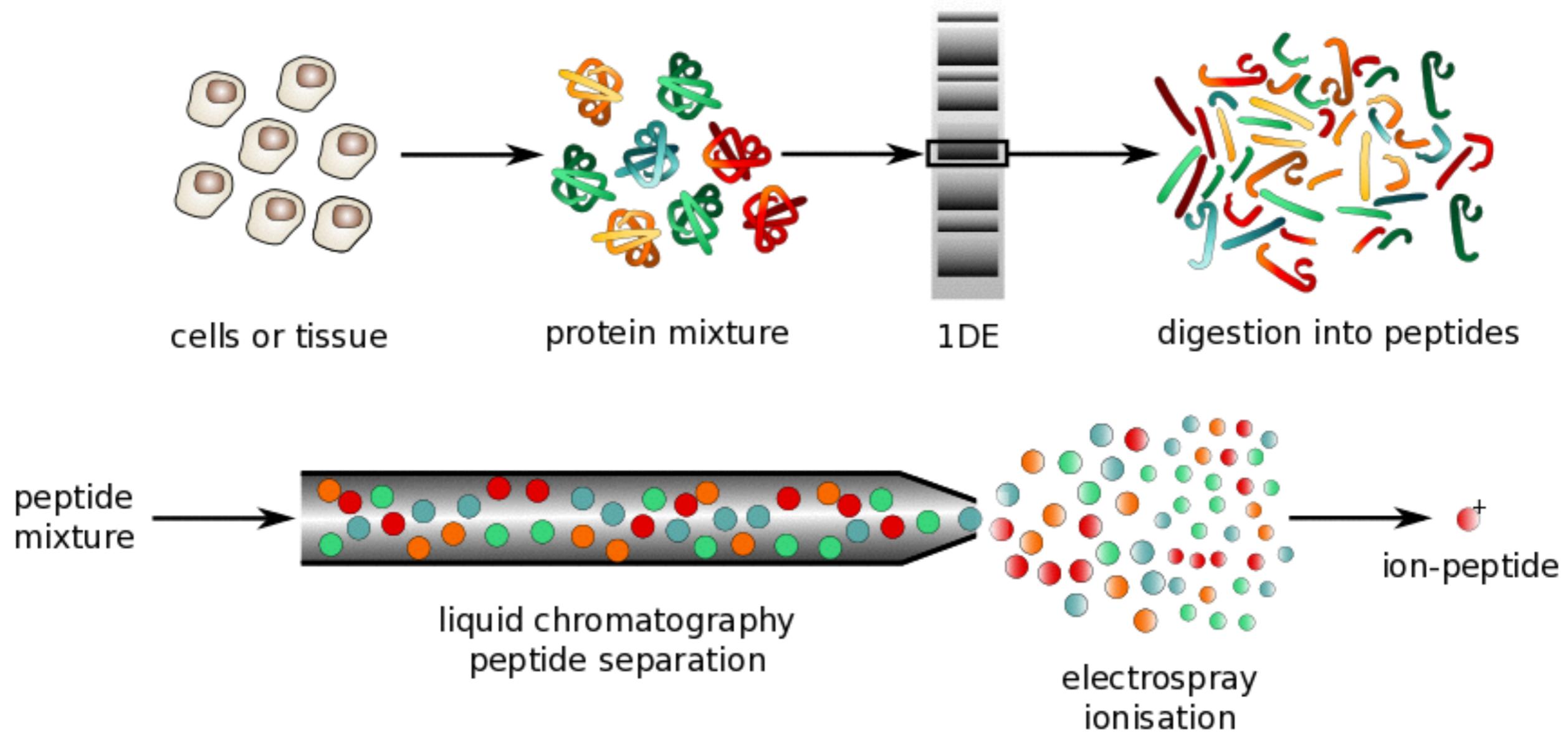


Citric Acid Cycle



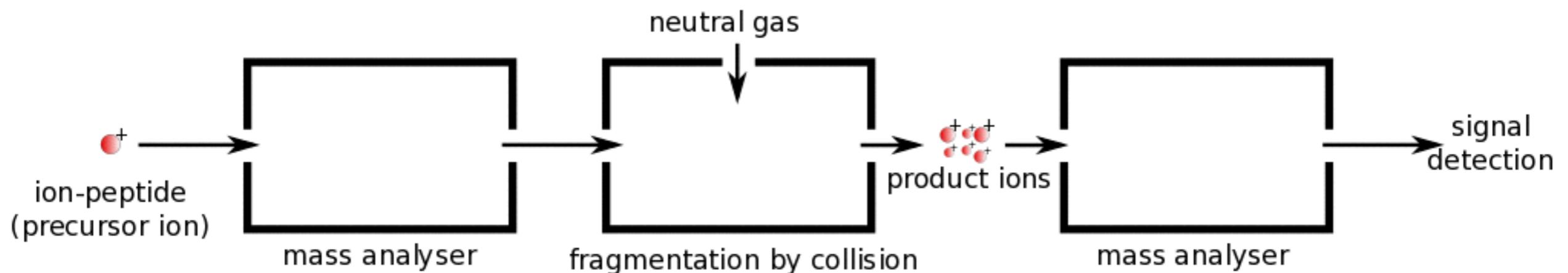


Mass spectrometry



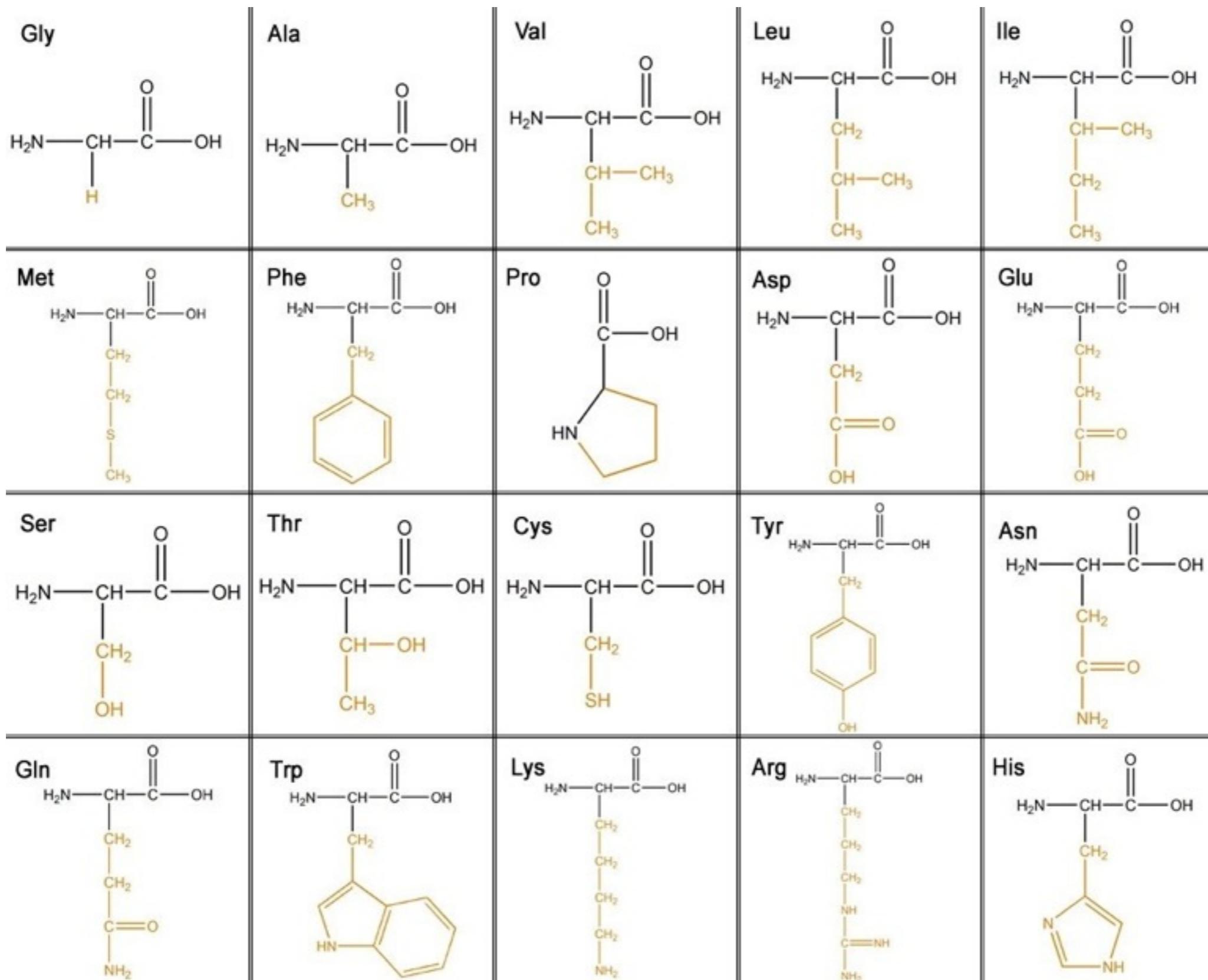


Mass spectrometry



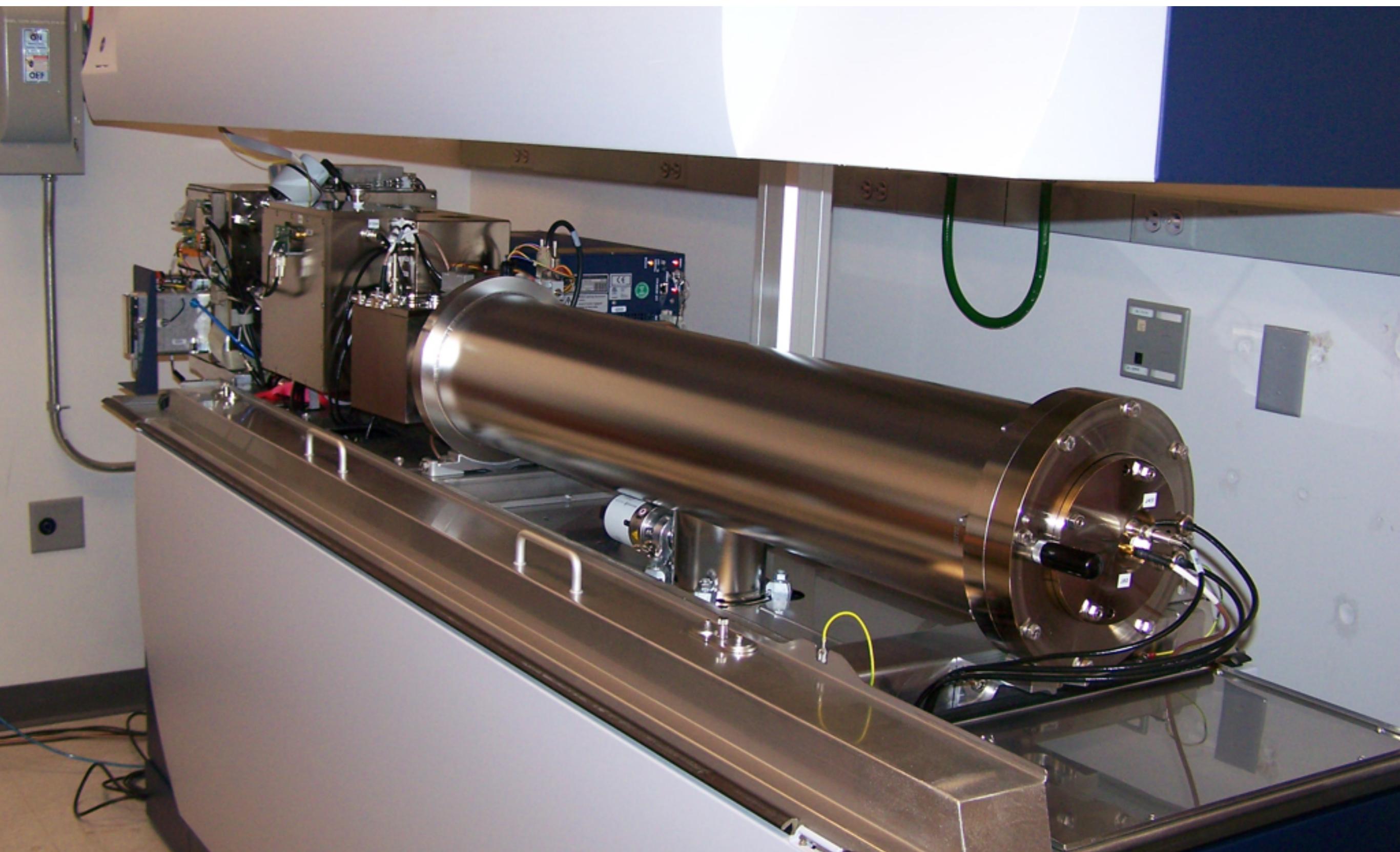


Amino acids, the building blocks



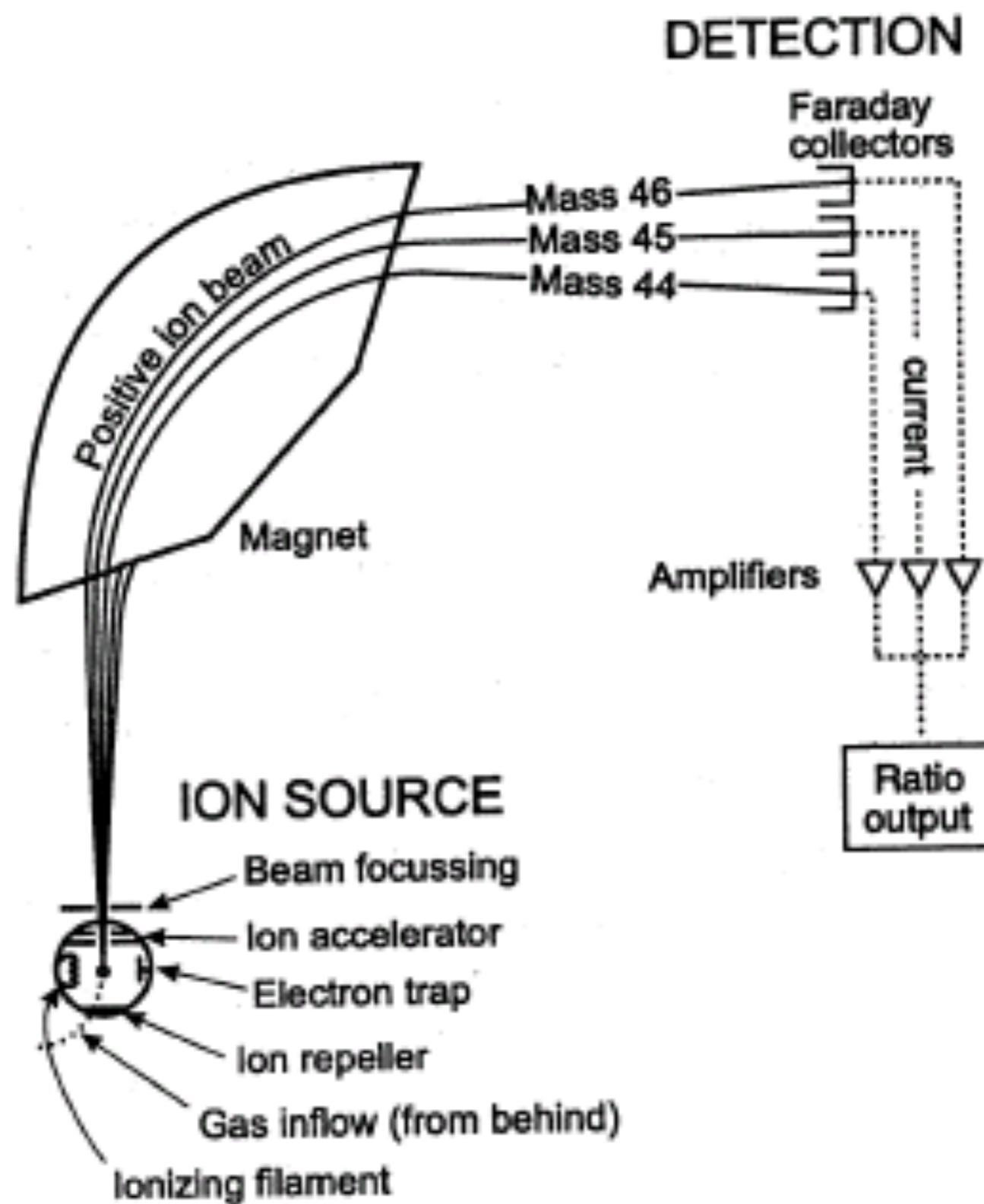


Mass spectrometer



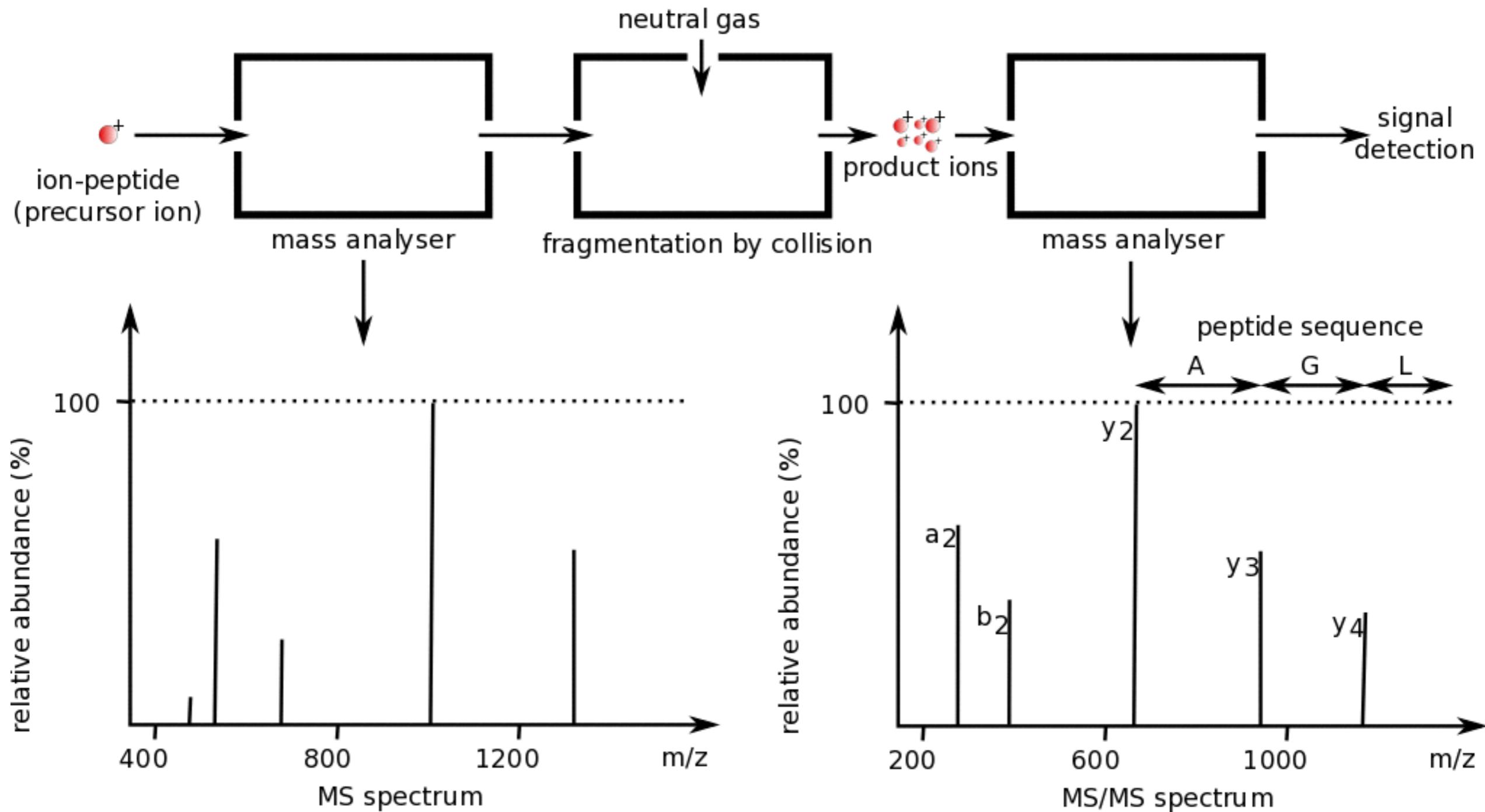


Inside the machine





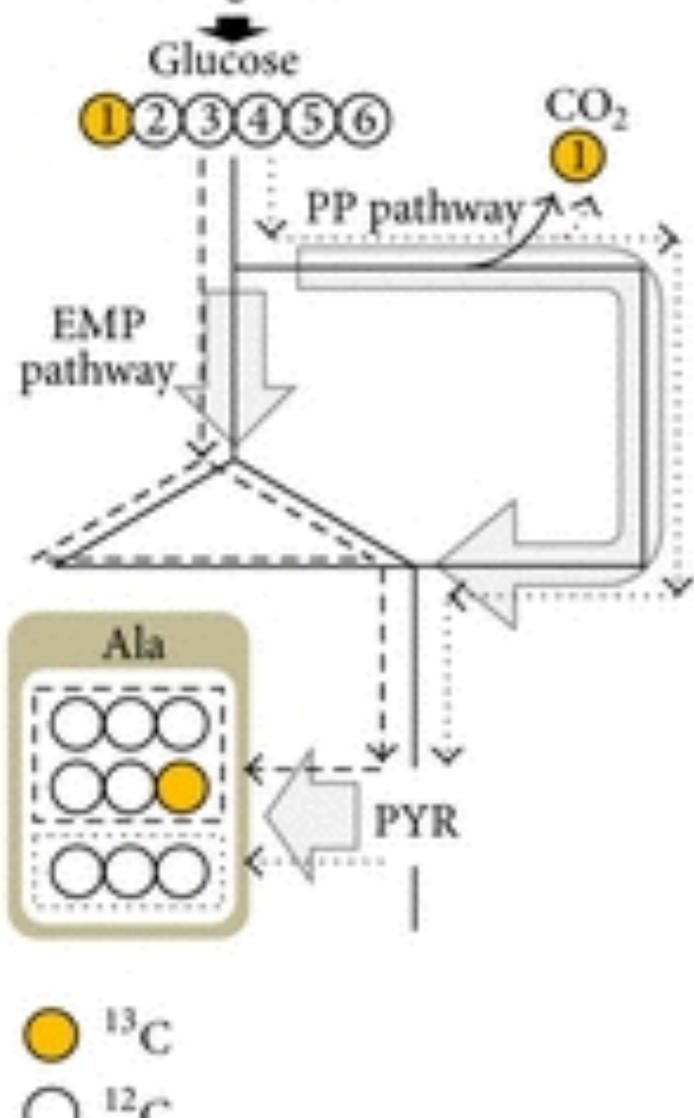
Mass spectrometry





C13 labeling

Cultivation with isotopically labeled glucose

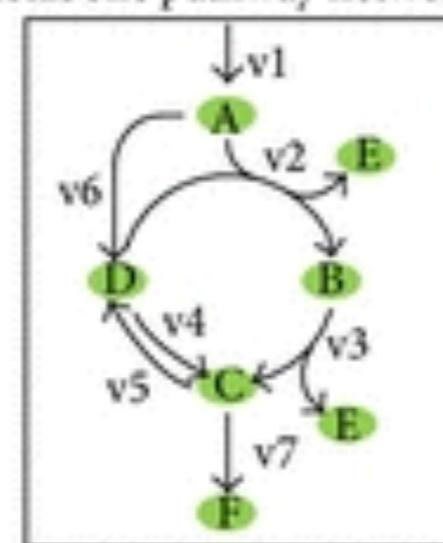


(a)

Metabolic.network.xlsx

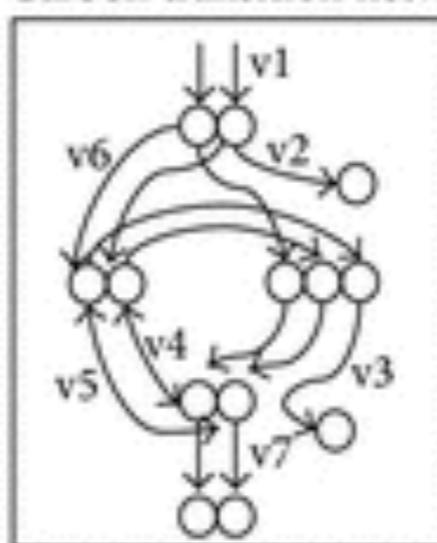
| FluxID | Rxns | NetFlux | Carbon.transitions |
|--------|-----------------|---------|--------------------|
| r1 | Sub_A → A | 0 | ab → ab |
| r2 | A + D → B + [E] | 0 | ab + cd → adc + b |
| r3 | B → C + [E] | 0 | abc → ab + c |
| r4 | D → C | 1 | ab → ba |
| r5 | C → D | 1 | ab → ba |
| r6 | A → D | 0 | ab → ab |
| r7 | C → [F] | 0 | ab → ab |

Metabolic pathway network

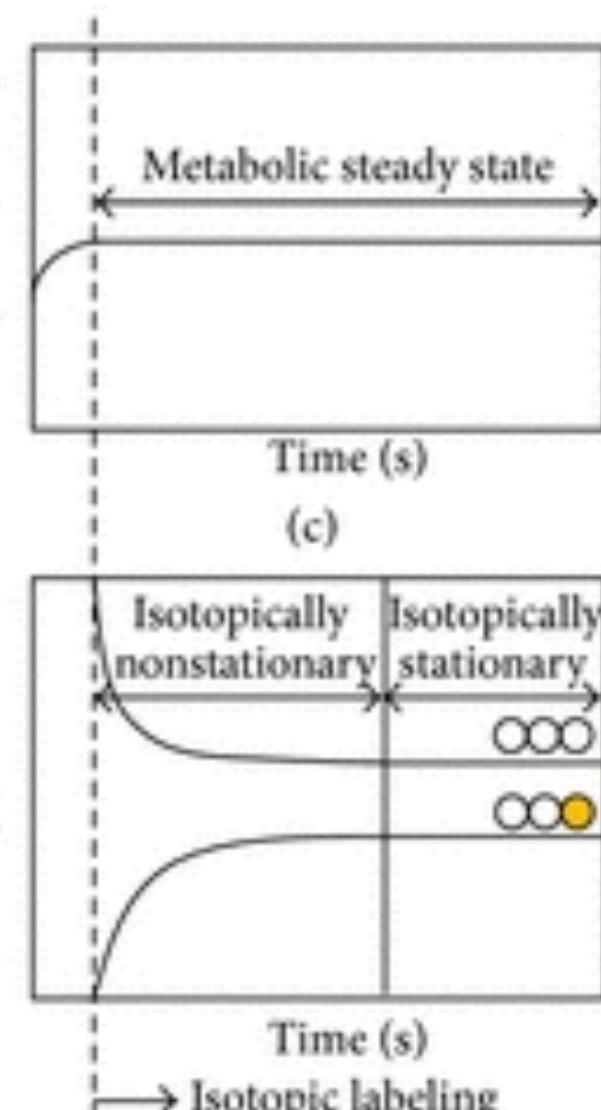


(b)

Carbon transition network



Mass isotopomer fraction Metabolite pool ($\mu\text{mol/gDCW}$)





Metabolic modeling

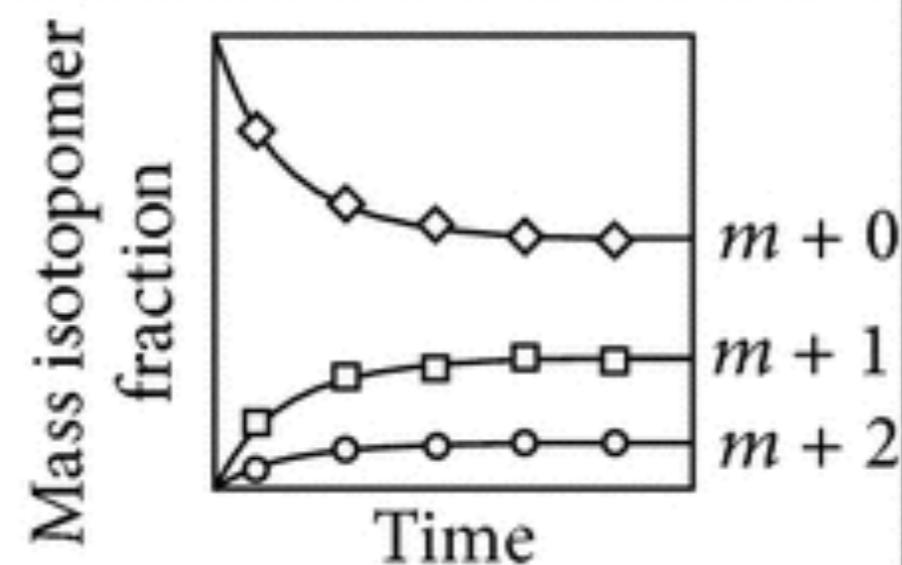
Metabolic model

$$\text{MDV}_{j,t=t_k}^{\text{sim}} = M(\nu, x^{\text{inp}}, X, t_k) \\ \text{s.t. } \text{SV} = 0$$

Experimental data

Biomass synthesis rate
Substrate consumption rate
Production rate

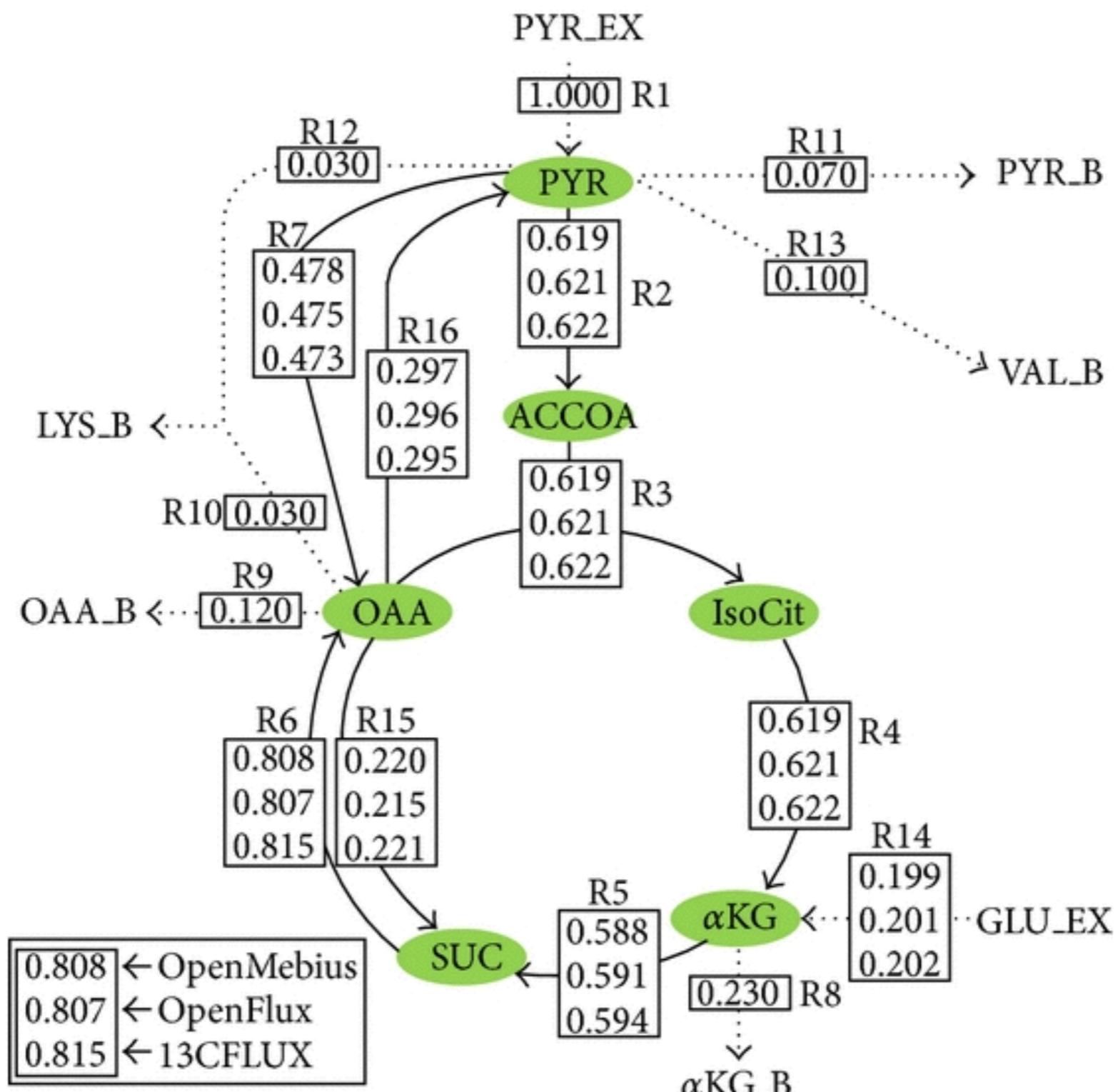
Step 1: initial estimates of metabolic fluxes are given following mass constraint



Experimental data
Metabolite pool



Flux estimations





MFA Software

- 13CFLUX
- 13CFLUX2
- C13
- Metran
- FIA
- influx_s
- OpenFLUX



some
rights
reserved