Hieronymous Bosch (ca. 1450–1516) - Garden of earthly delights painted in 1503-1504; on display in Museo del Prado in Madrid

concept of intestinal flora

Use of ^{13}C-labeled substrates to decipher fermentation of prebiotics in the human colon *in vitro* and *in vivo*

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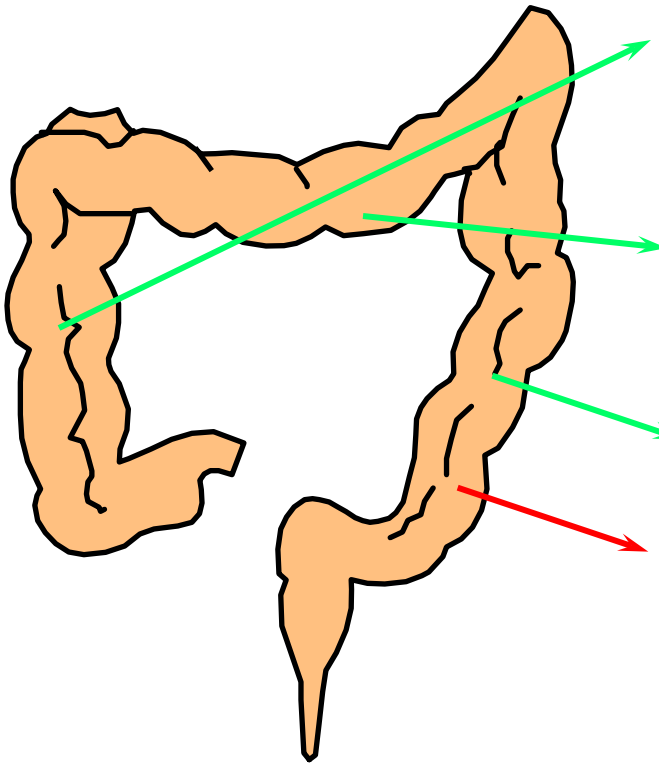
Carbohydrate fermentation in the colon

Who does what?
What is made?
What is the effect on health?



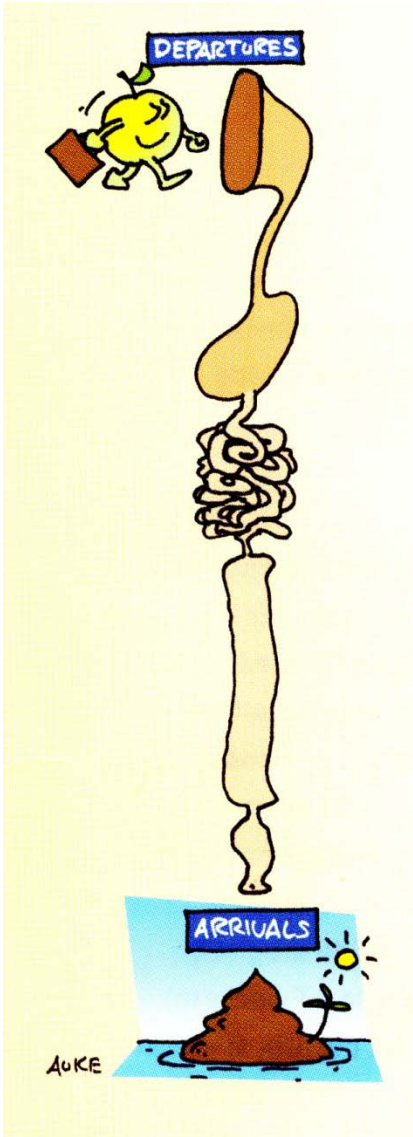
proximal colon: primarily saccharolytic fermentation

transverse colon: combination of saccharolytic and proteolytic fermentation

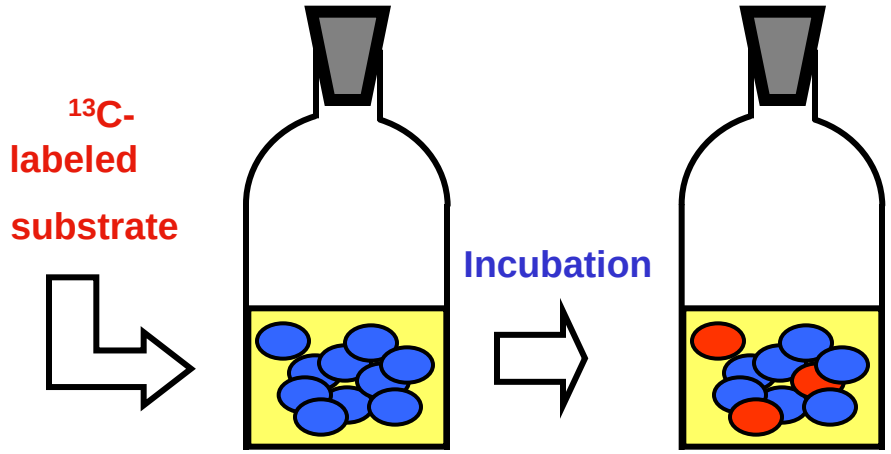
distal colon: primarily proteolytic fermentation

distal colon: colon cancer and IBD

Limited accessibility of the colon



Principle: Use of stable isotopes



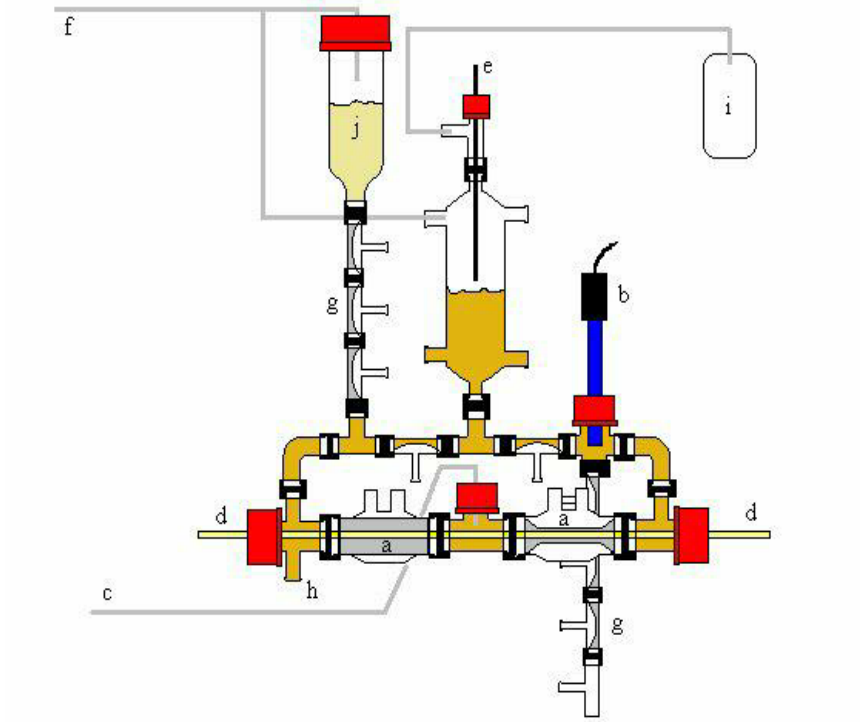
- ^{13}C labelled model substrates
  - lactose
  - inulin
  - starch

^{13}C ends up in microbial metabolites

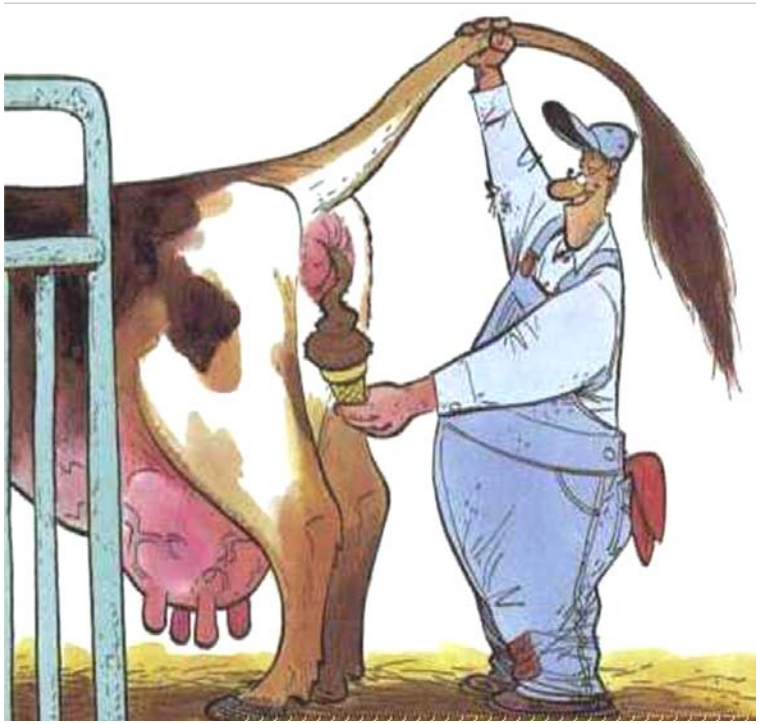
^{13}C ends up in microbial biomass (incl. DNA/RNA)

De Graaf and Venema, AMP 2008

In vitro model of the colon: TIM-2



the *in vitro* model



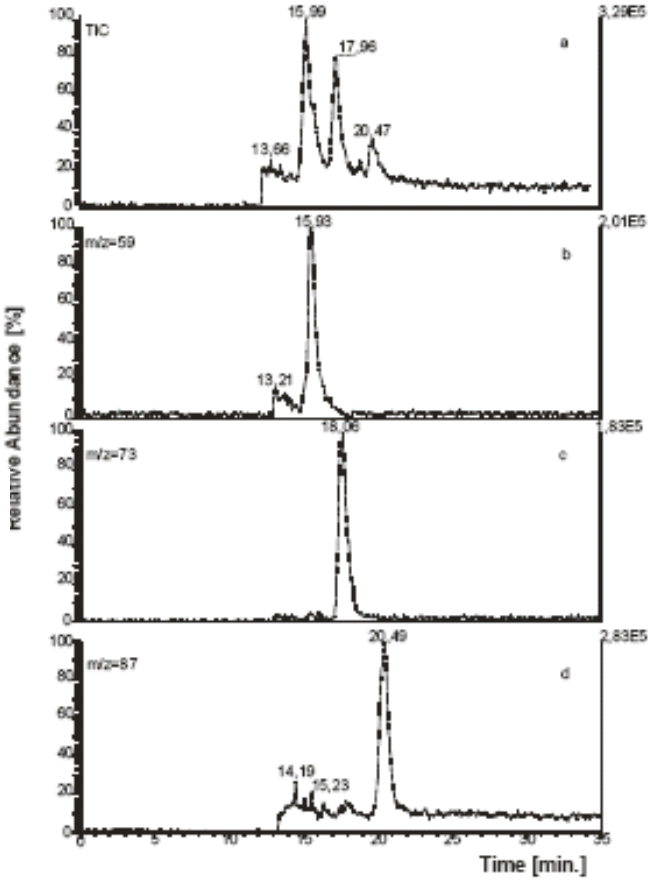
the inoculum

Analyses

^{13}C isotopomer analysis:

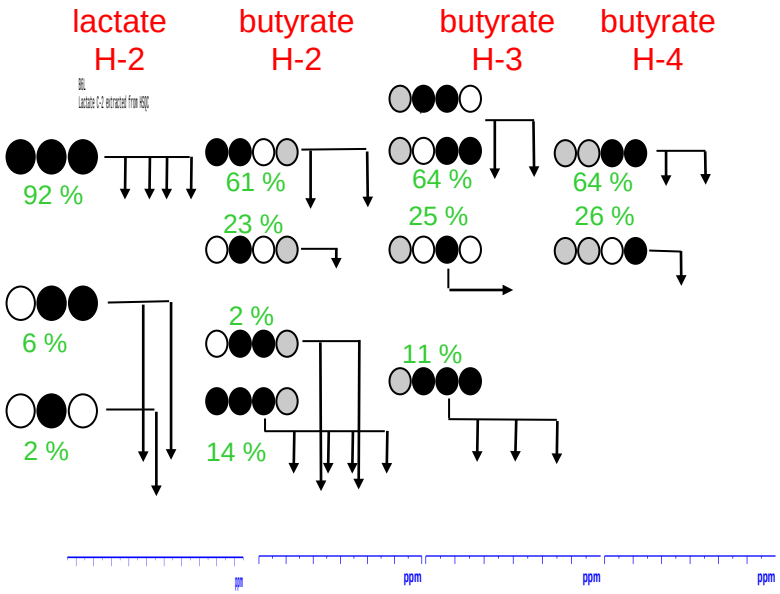
LC-MS

more sensitive

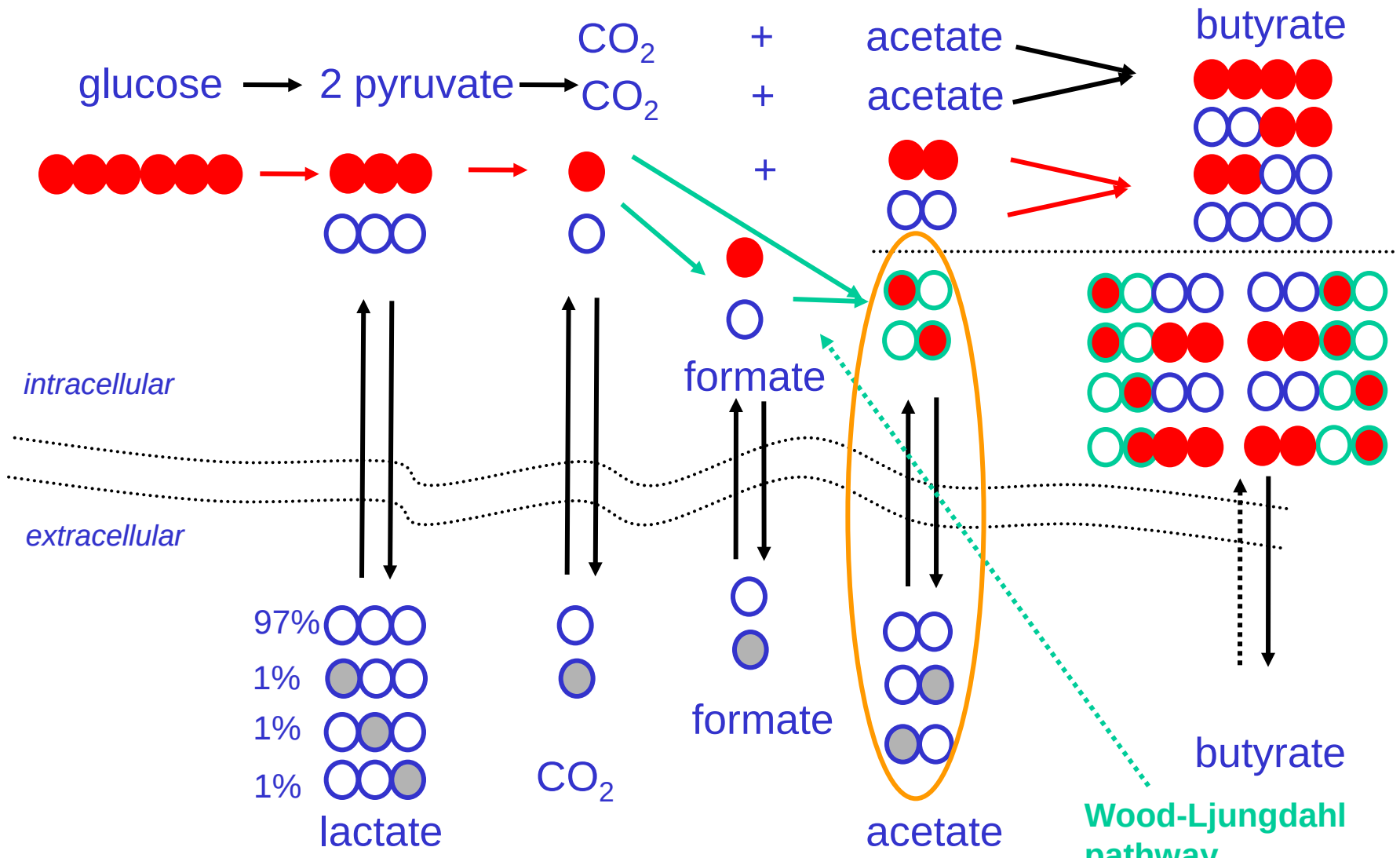


NMR

positional data



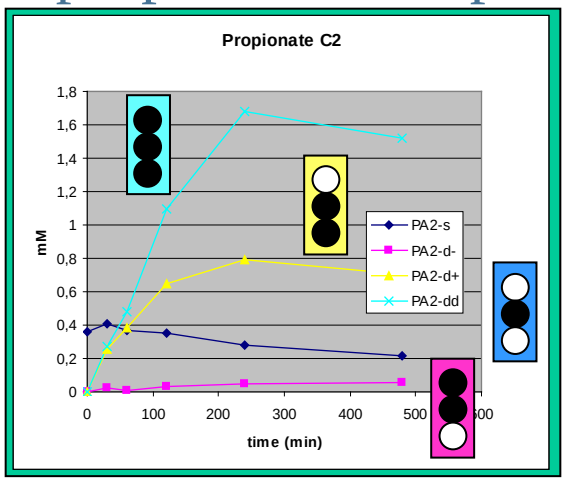
Bacterial metabolism



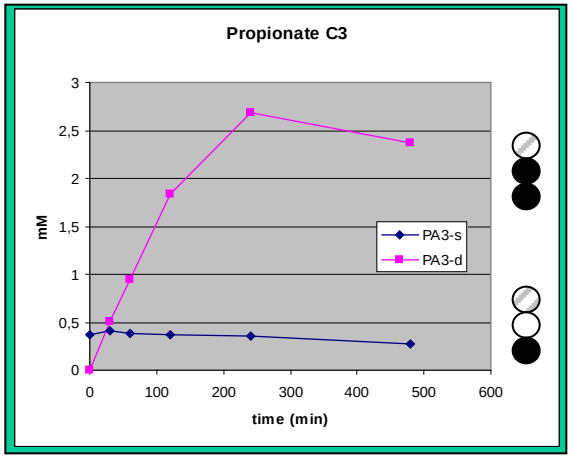
Propionate – starch experiment

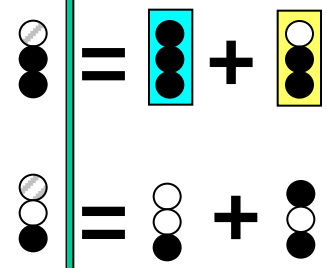
^{13}C propionate isotopomers

NMR

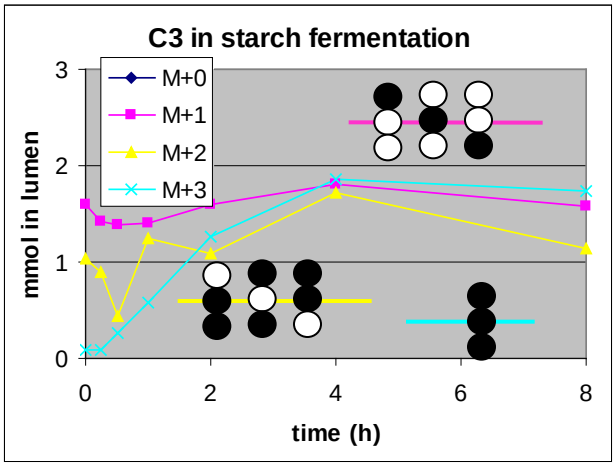


NMR

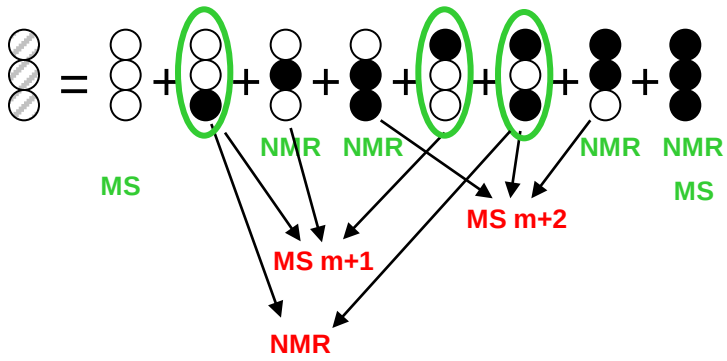




MS



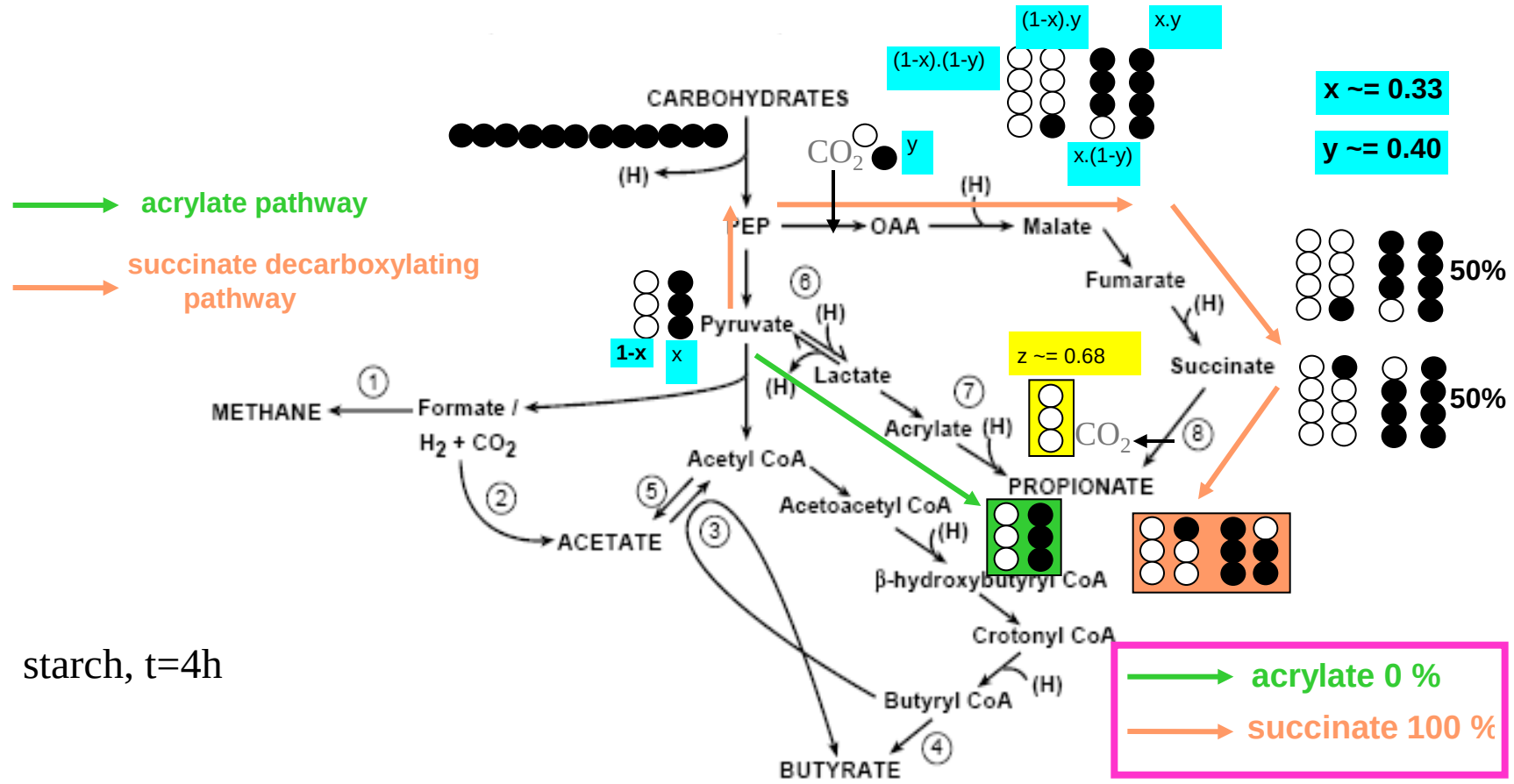
measurements



=> all propionate isotopomers determinable

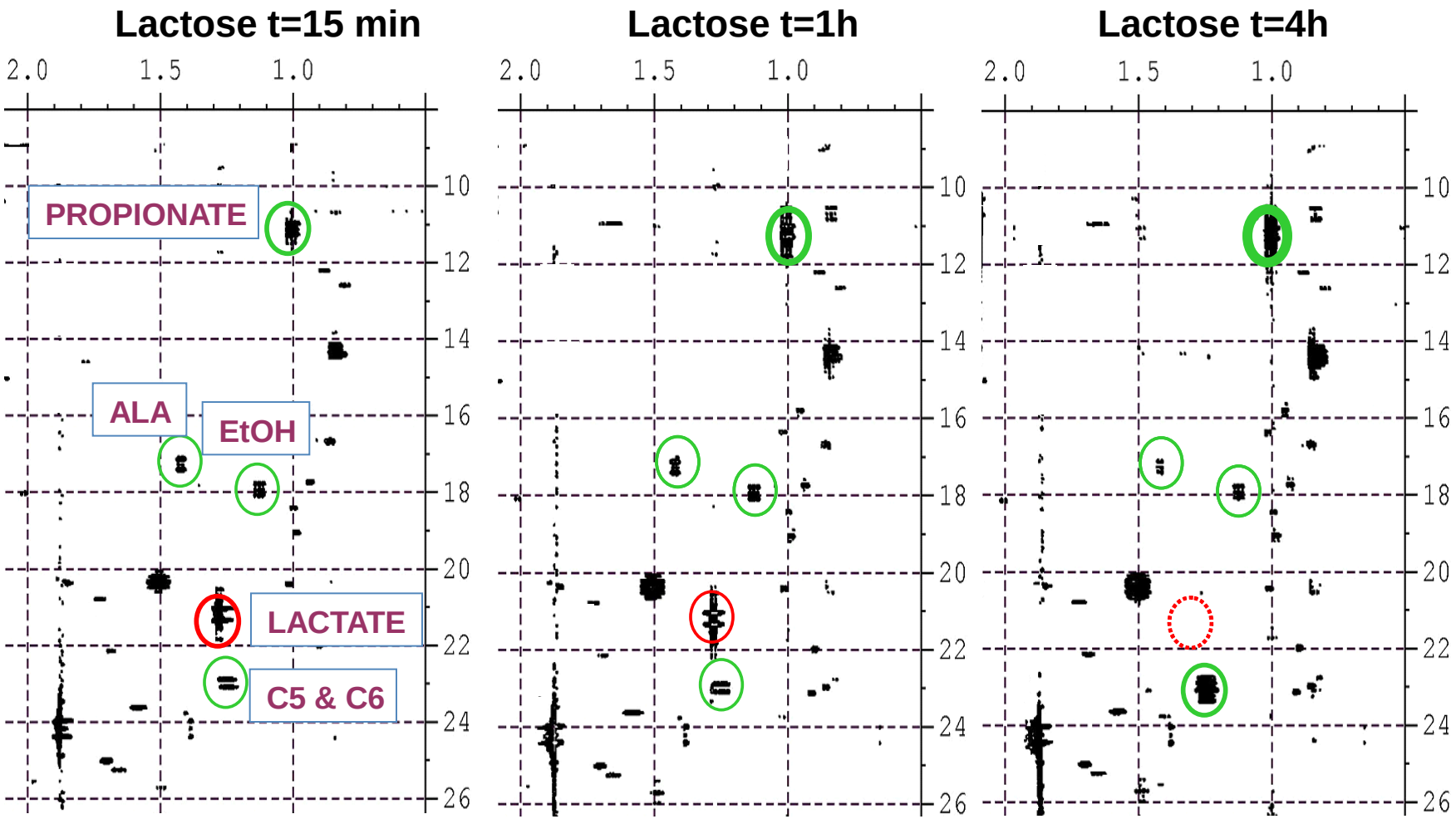
Propionate pathways

Acrylate and succinate-decarboxylating pathways

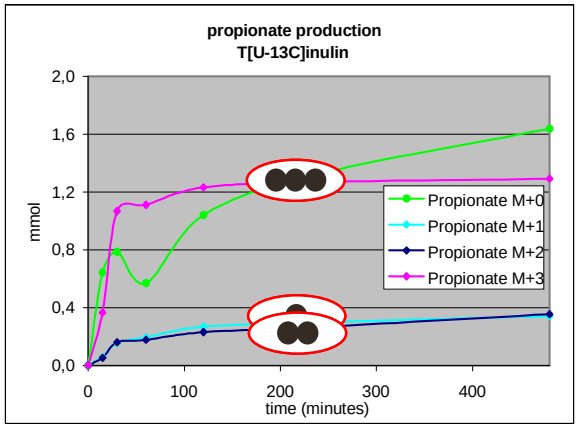


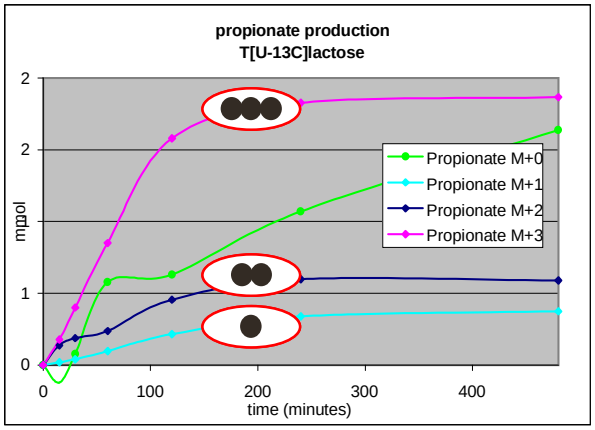
starch, t=4h

Propionate – lactose experiment



Propionate pathways





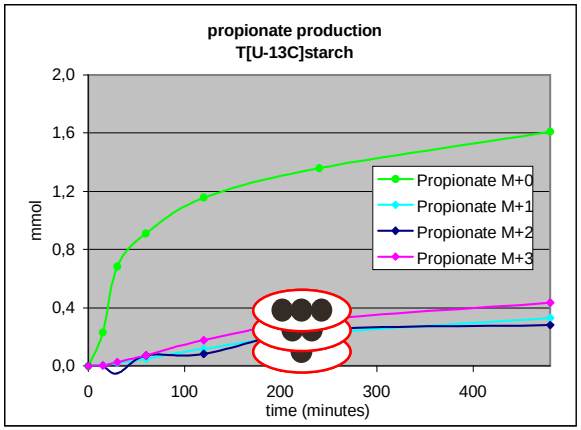


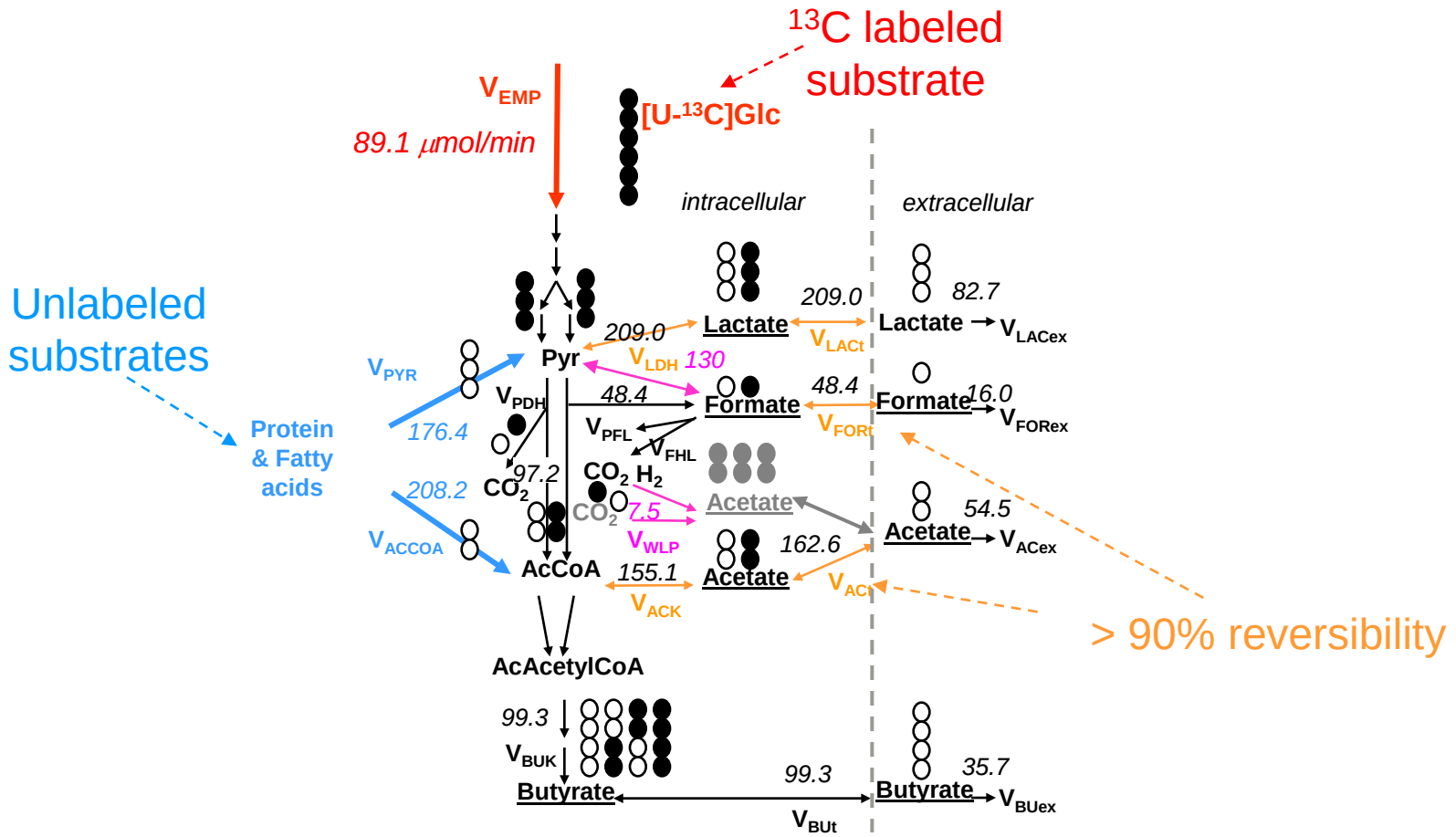
Table 2.2.1. Flux distribution over the propionate synthesis pathways on different ^{13}C-labeled substrates estimated from the isotopomeric labeling data.

substrate	acrylate pathway contribution (%)	succinate decarboxylation pathway contribution (%)
starch	0	100
inulin	60	40
lactose	50	50

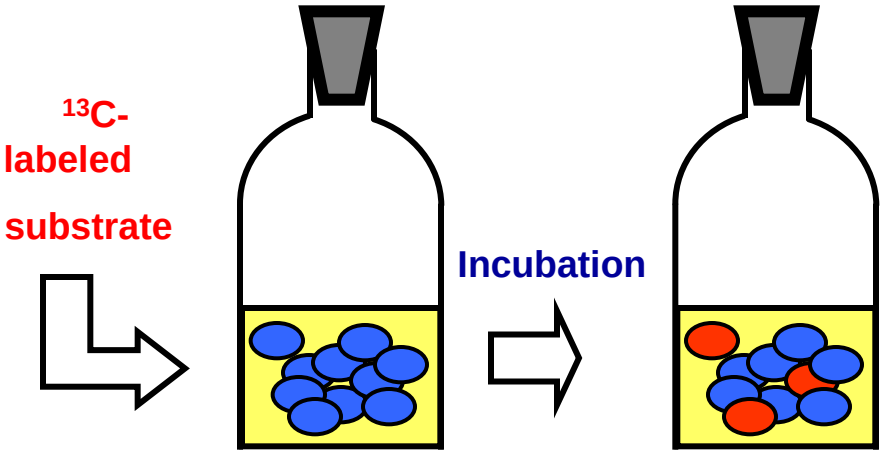
These results seem to indicate that a faster fermentation goes along with an increased contribution of the acrylate pathway, i.e. the pathway that has lactate as a precursor.

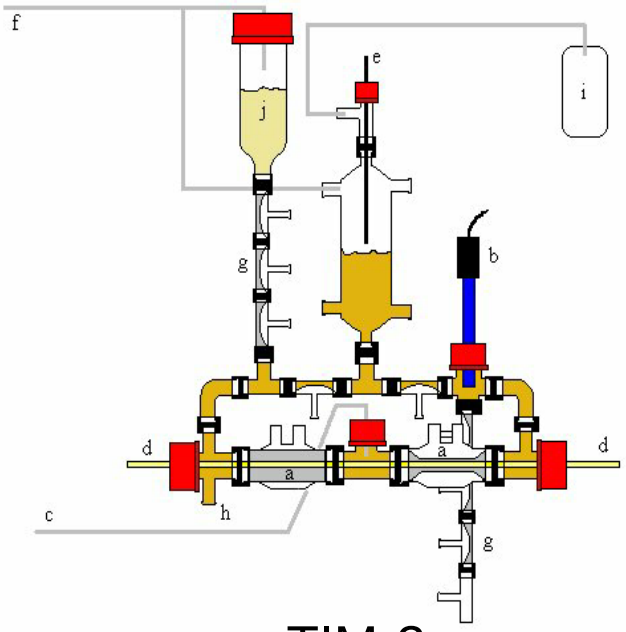
TIM-2 [U-^{13}C]-starch experiment

Computer model: Flux within intestinal microbiota



Principle:
Use of stable isotopes





TIM-2

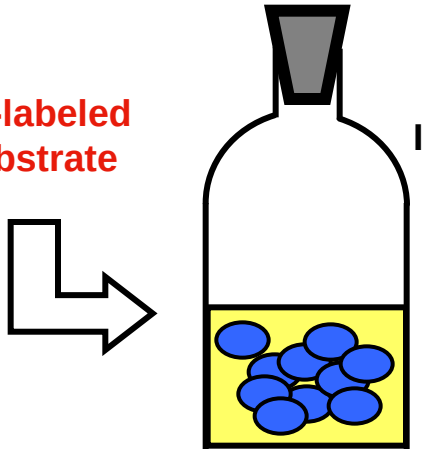
^{13}C ends up in microbial metabolites

^{13}C ends up in microbial biomass (incl. DNA/RNA)

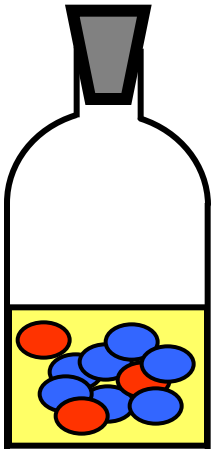
De Graaf and Venema, AMP 2008

16S rRNA-SIP

^{13}C-labeled substrate

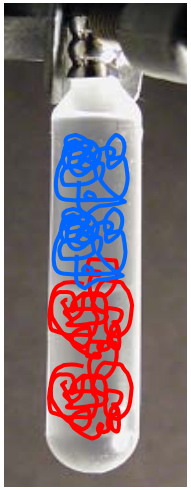


Incubation

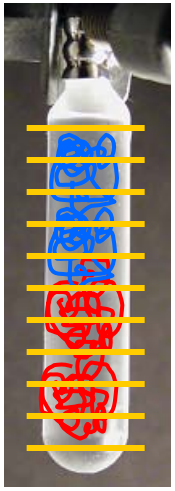


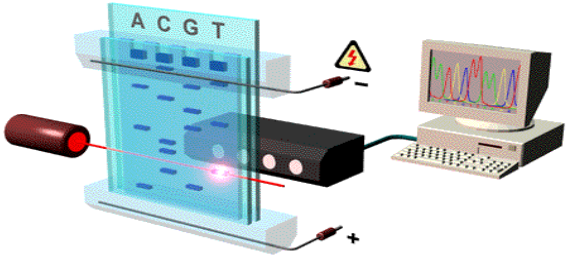
RNA extraction

Density gradient ultracentrifugation (CsTFA)



Gradient fractionation

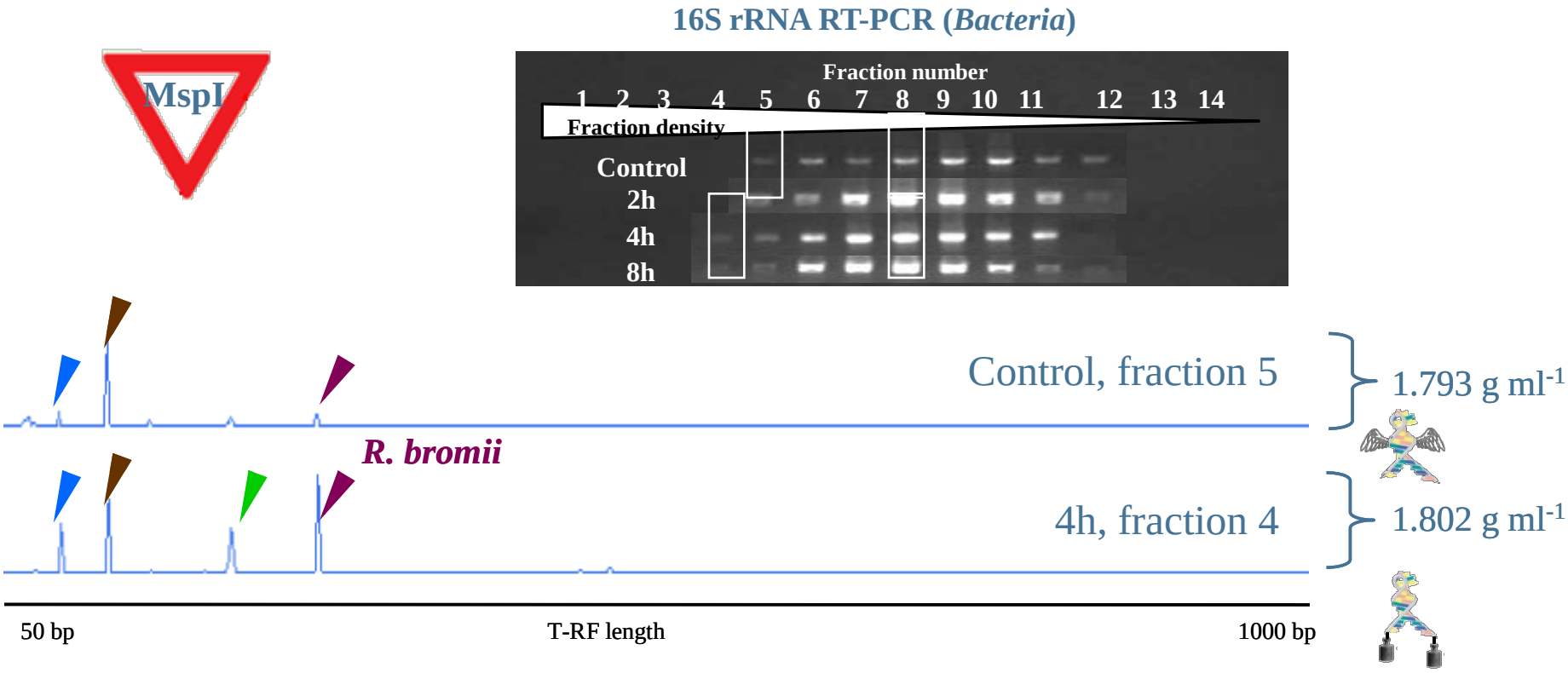




Molecular analyses heavy vs. light fractions

- ✓ 16S rRNA - based molecular fingerprinting of microbial diversity
- ✓ Cloning and sequencing of 16S rRNA

Which microbes enjoy the “heavy” meal?



Fermentation products 8h after starch addition:

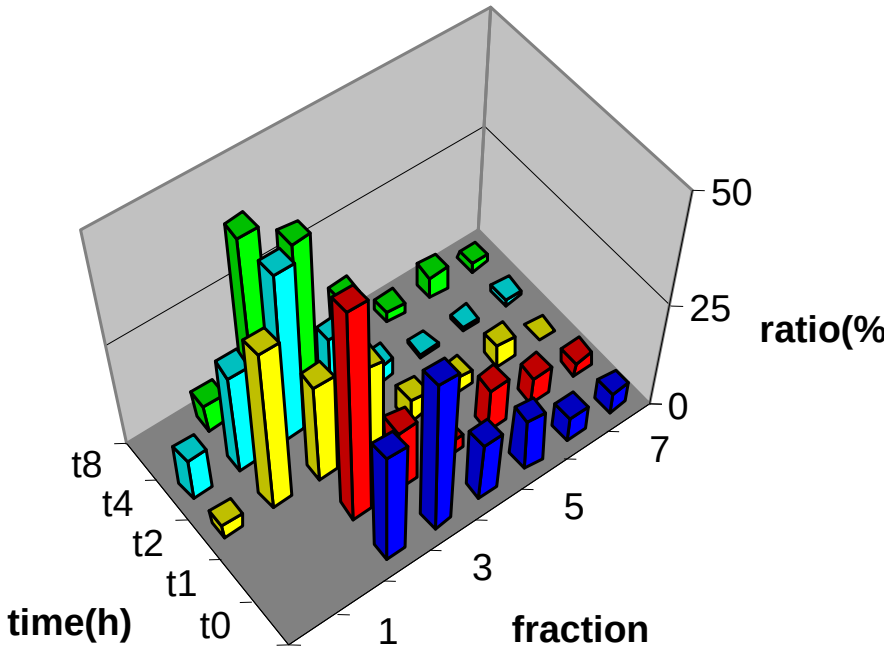
acetate > butyrate > propionate

Ruminococcus bromii > *Bac./Prev.*spp → **acetate** / **propionate** → *Eubacterium rectale* → **butyrate**

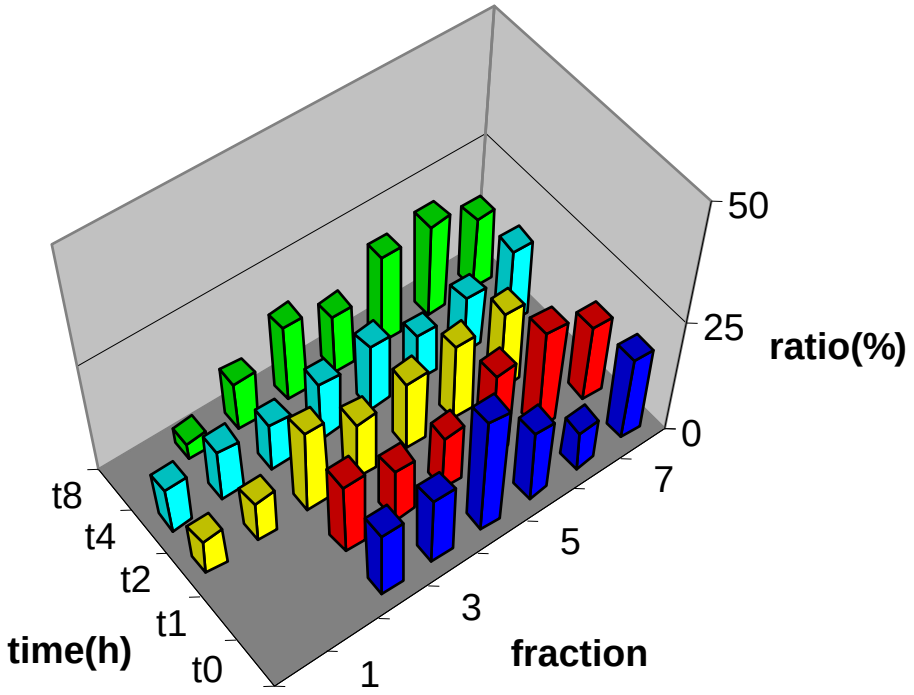
Kovatcheva-Datchary et al. (2009), Environ Microbiol

Use of GOS by the intestinal microbiota - Implications for claim substantiation

Bifidobacterium bifidum



Lactobacillus gasseri

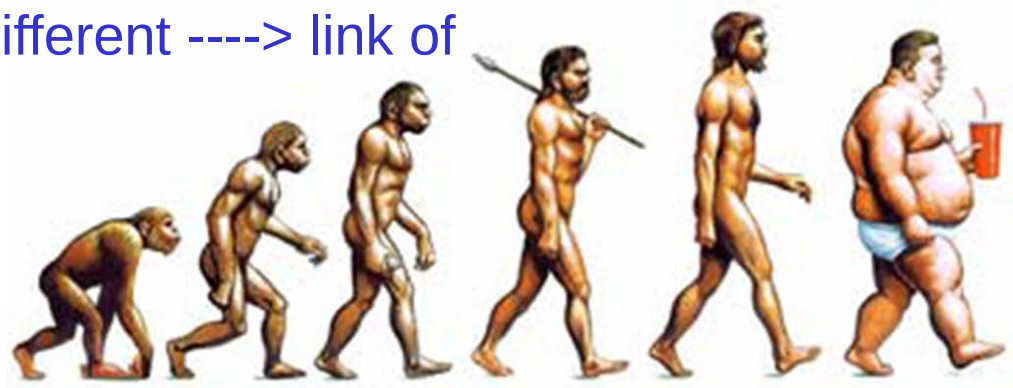


Maathuis et al., (2010) in preparation

Determination of caloric value - Implication for obesity

Metabolite concentration in mmol and energetic value of **1 g inulin:**							
	m+1	m+2	m+3	m+4	sum	kcal/mol	kcal
acetate	0.362	4.317			4.679	209	0.978
propionate	0.125	0.392	1.868		2.386	367	0.876
butyrate	0.043	0.302	0.000	0.026	0.372	524	0.195
Energetic value of 1 gram inulin (in kcal):							**2.05**

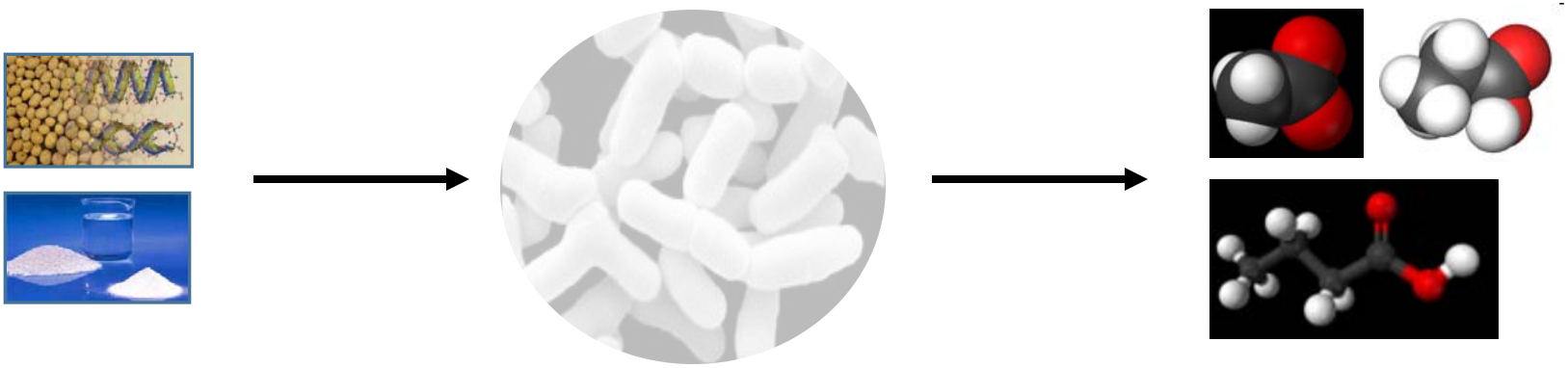
- 1 gram of inulin has a caloric value of 2.05 kcal
- similarly, 1 gram of lactose has a caloric value of 2.41 kcal
  1 gram of starch 1.69 kcal
- Therefore, depending on the fermentable substrate, the energy-harvest of the body is different ----> link of microbiota with obesity



Venema (2010) Curr Opin Clin Nutr Metab Care

Conclusions

- Stable isotope-labeled substrates are excellent tools to study the processes occurring in the inaccessible colon - even in human individuals
- The label can be traced in metabolites and microbial biomass
- This allows to create the food-chain in the gut:



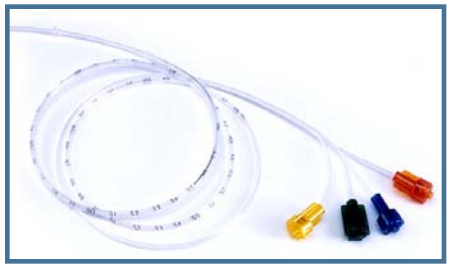
substrate microbe metabolite

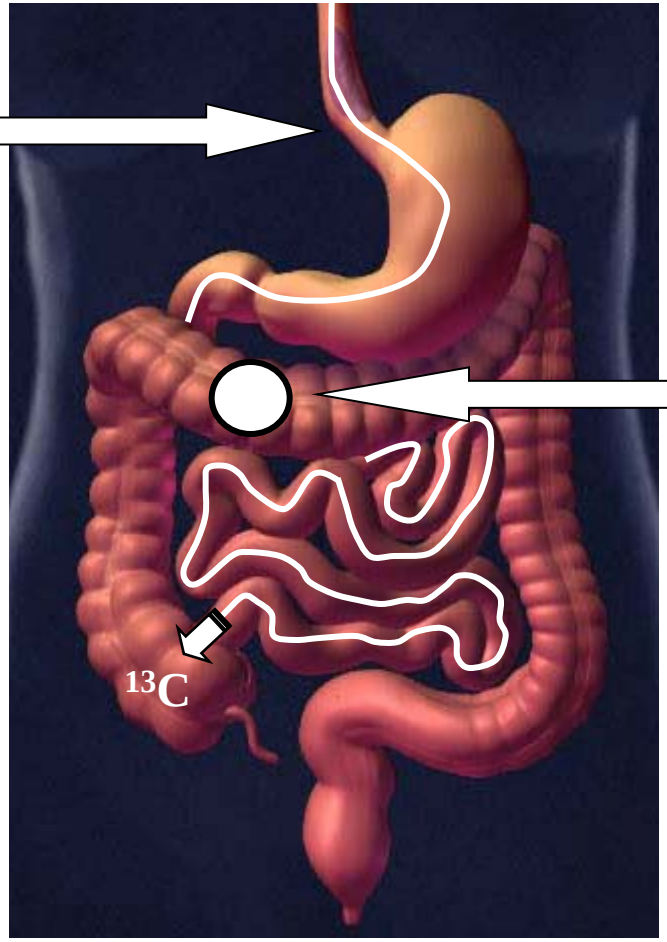
- Precise determination of the metabolites produced allows the exact calculation of energy harvested from fermentable substrates

Outlook

human feeding trials @ Maastricht University hospital

Substrate delivery via a nasal tube



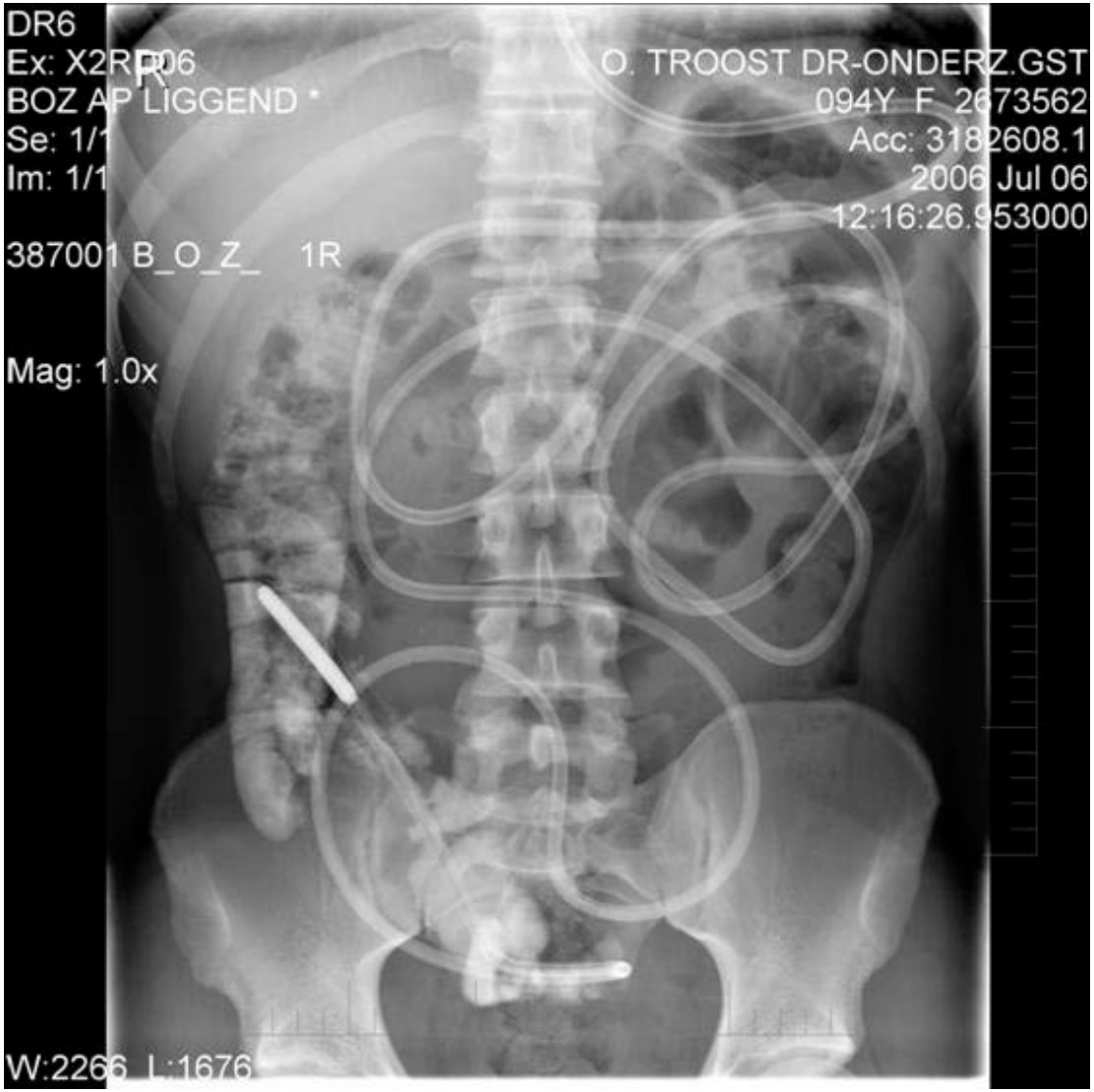


Colon sampling via a stoma in the *colon transversus* or back through catheter

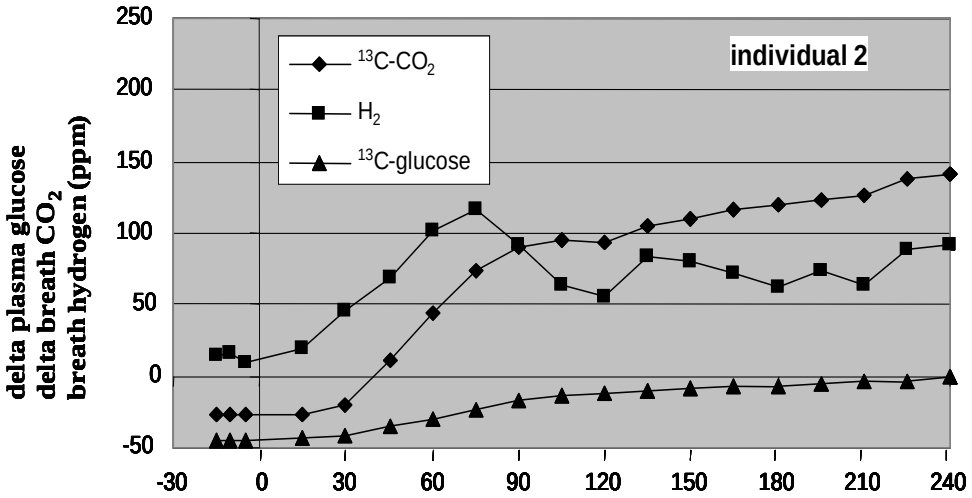
Plasma sampling

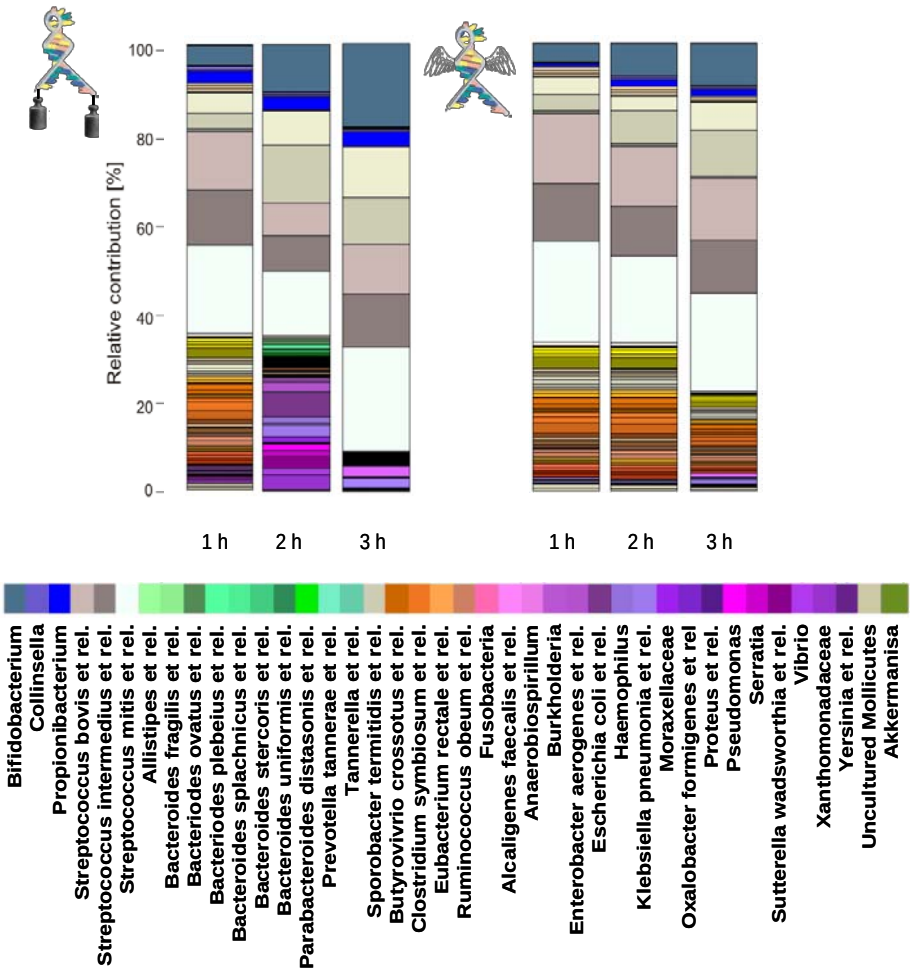
LC-MS analyses

First experiments in humans!!



Use of the nasal catheter in 2 healthy volunteers





- no ^{13}C-glucose in plasma
- increase in ^{13}C-CO_2 in breath
- in crease in breath H_2

↓

fermentation of ^{13}C-lactose in colon

Acknowledgements

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TNO

WAGENINGEN UR
For quality of life

rijksuniversiteit groningen

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