

Farm animal proteomics- from a Systems Biology perspective

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Hinxton



AARHUS UNIVERSITET

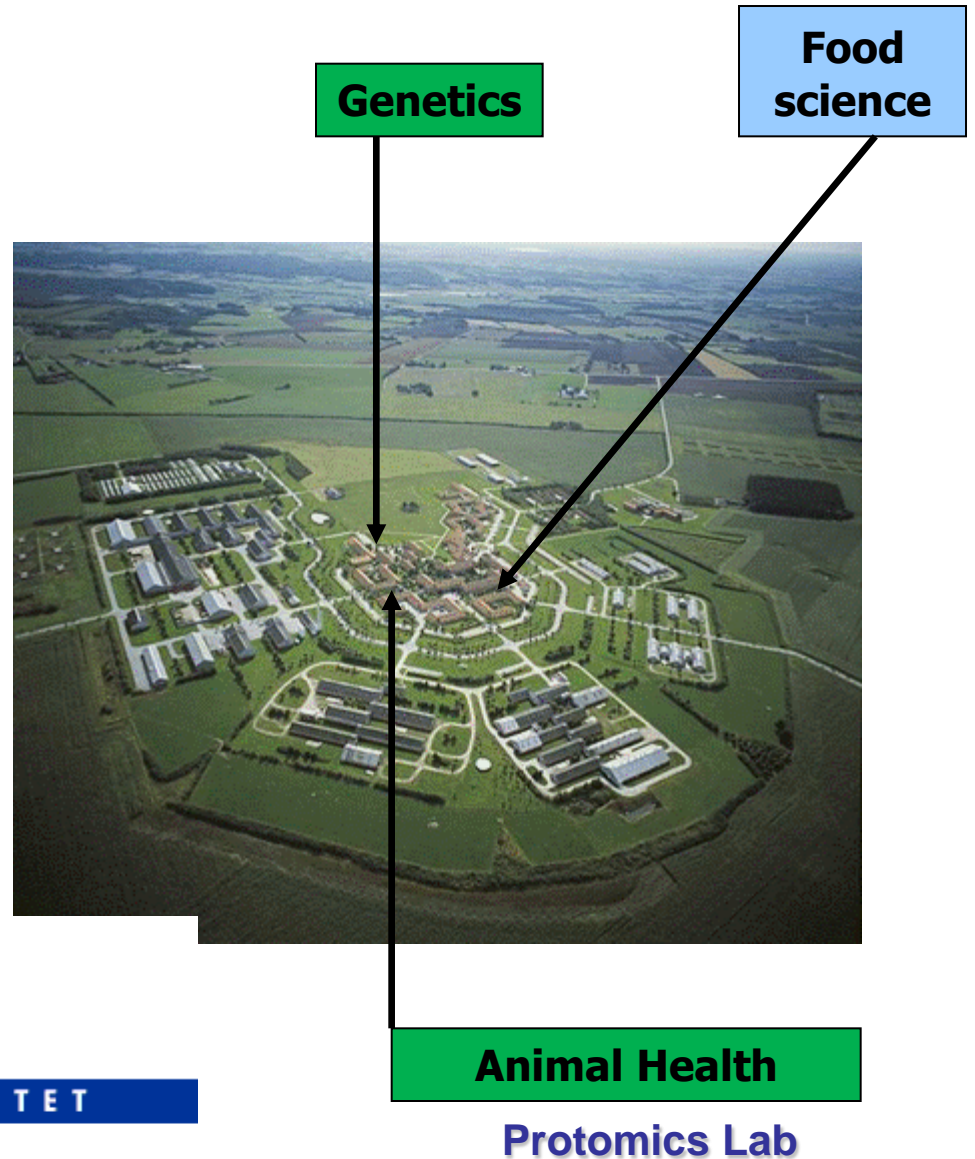
Faculty of Agricultural Science

Off- Campus RC

- 600 colleagues
- >2000 animals

Institutes:

- Genetics,
- Animal Health and Biosciences
- Food Science



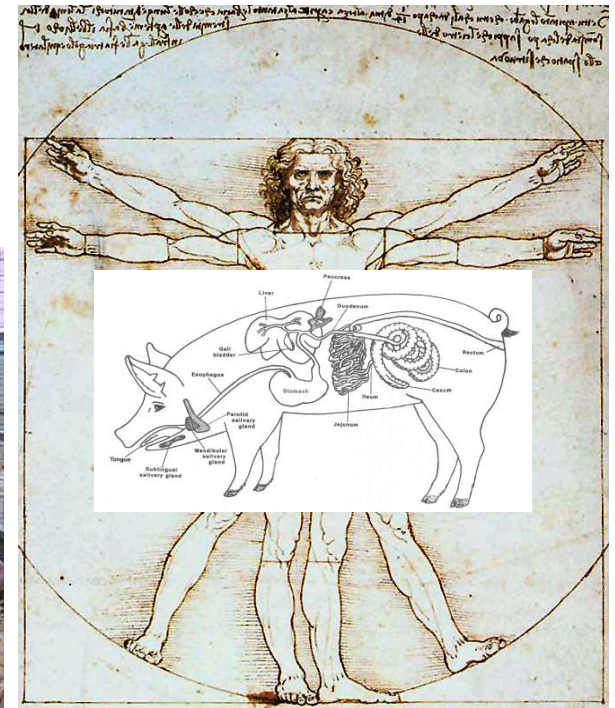
Proteomics in farm animals

Biomarkers for :

Solving the problems of farm industry

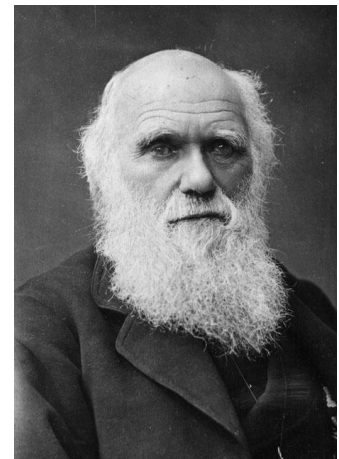
- **Health**
- **Growth**
- **Fertility**
- **Milk** (yield and quality)
- **Meat** (yield and quality)

Model organisms for human biology

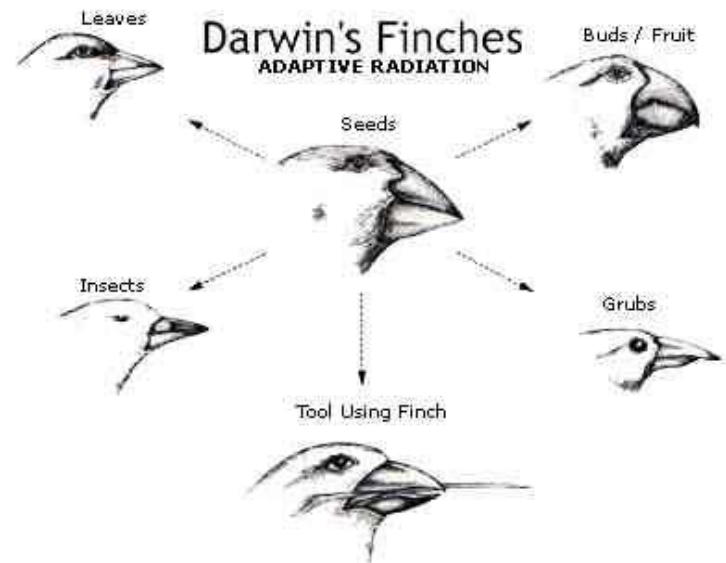


Studying biological variation-

the key to characterising
biological systems



- Biologic variation through **natural selection**



Biological variation in Farm animals

- originates from **selective breeding**



Breed: Jersey

Milk Production (50 L/day)

Biology: complex genetic influence (remains to be characterised)



Breed: Belgian Blue

Meat production (5Kg muscle/day)

Biology: Myostatin gene mutation

Biological variation in Farm animals - originates from **selective breeding**



Breed: Danish Landrace

Meat production

Lean growth



Breed: Hungarian Mangalica

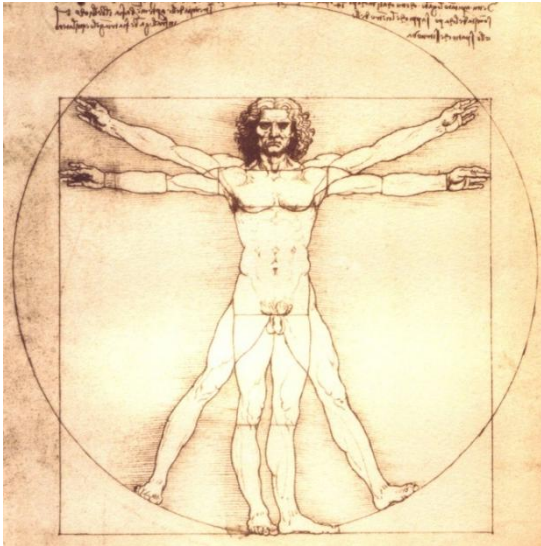
Fat production

Obese growth

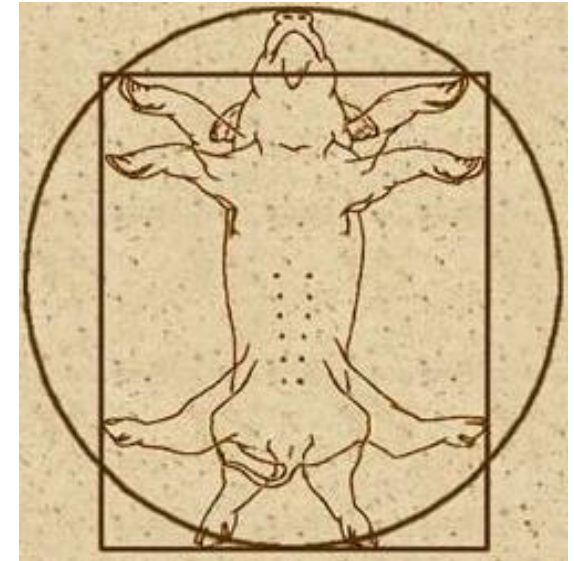
Model animals

Farm animals are very special sets of biological samples, that allow studies of extreme physiology

Pig as a model organisms for human disorders:



Genome homology
Metabolism (omnivore)
Brain anatomy
Body size
Organ size
Gut physiology



Genetic variation
intensely studied
and well
documented

Pig production...25 mio/year
(DK)

Documentation

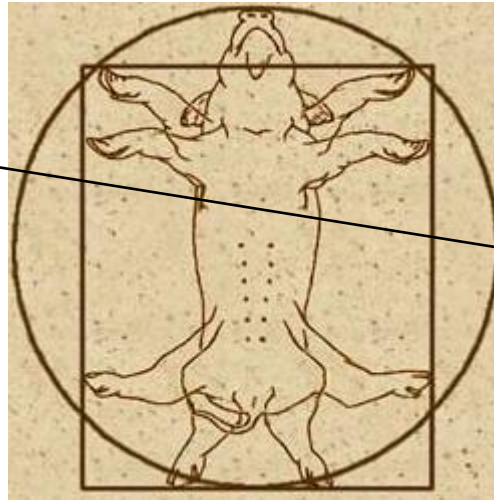
Book keeping

Lines of breeds...variation

Our pig genome resources

Trait-genome mapping

- Genetic variation in a 12,000 animal family (**12 boars**)
- >20 tissues collected
- Growth traits **linked to genetic variation (SNPs and QTLs)**
- Metabolism
- Obesity-fat deposition
- Lean growth



Genome

Complete, but still not fully annotated-

4 genomes completed

Transcriptome

- cDNA arrays (27K)
- Oligo-arrays (24 K)
- mRNA quant (454-reads)
- Tag based (Sage-Solexa)
- Small RNAs (Solexa-454)

SNPs

- 50 K arrays

Source of animal models

Transgens

- Parkinson (a-synuclein)
- ALS (SOD1-G93R)

Today's talk:



Proteome
studies related
to **gut health**
and **metabolism**



Proteome
studies
related to
mammary
gland health

Gut health in Industrial pig production

- 25 million pigs/year (Denmark)
- Neonatal mortality 5-10%
- 90% deaths → 1st week
- Mostly related to "gut problems"
- An antibiotics-issue (the resistance problem)



- **Ethical and economical problem !!!!!**

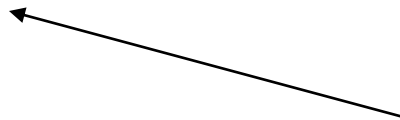
Host bacteria interactions in gut

Functions of gut epithelium

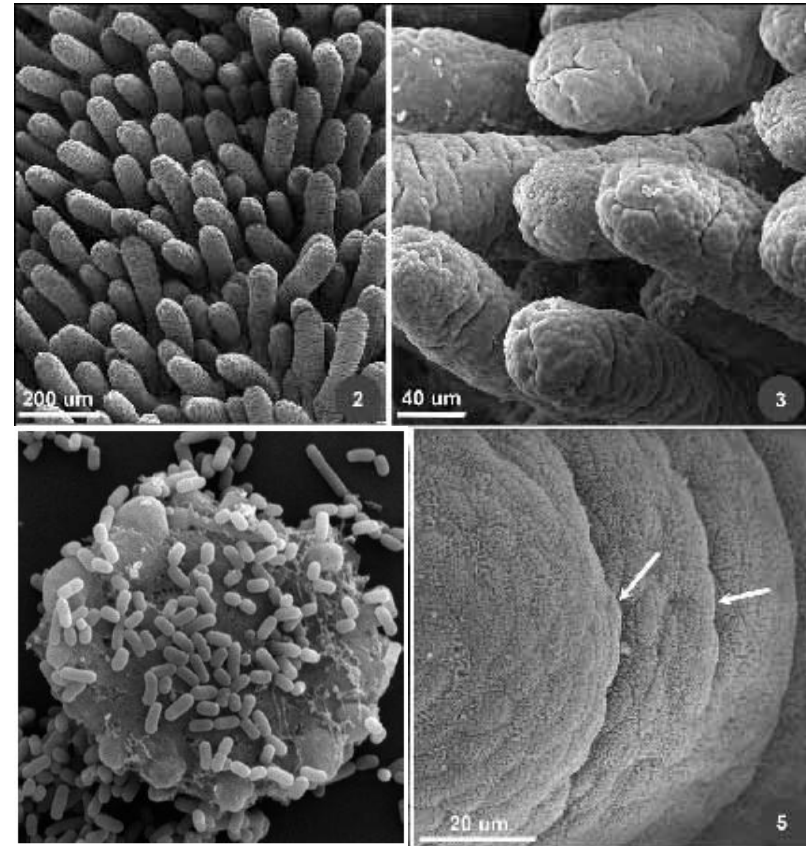
- Nutrient uptake
- Frontier
- Defense
- Knowing friend from enemy



Gut cell plasticity



Controlled by genes and environment!

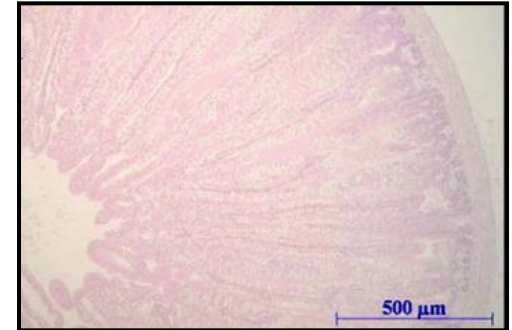


Phenotypes in germ-free animals

Gut morphology and function

Villi are longer and thinner

Capillary networks are reduced



Metabolism

Extract less energy from diet

Lipid metabolism altered

Immunology

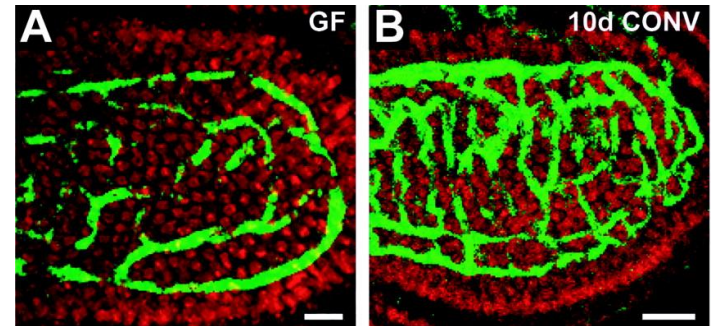
Reduced secretion of IgA

Smaller Peyer's patches

Reduced number of M-cells

Decreased production of antimicrobial proteins

Impaired regulatory T-cell development



Gnotobiotic pig model

Does different **gut bacteria** have different impacts on gut **tissue development**?

12 germ-free piglets

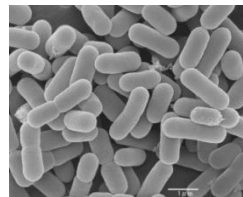


4 x



Maintained germ-free

4 x



Lactobacillus fermentum

4 x



Escherichia coli

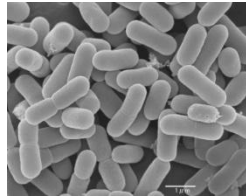
Gnotobiotic pig model

Does different **gut bacteria** have different impacts on gut **tissue development**?

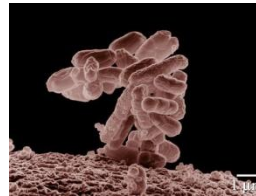
Maintained
germ-free



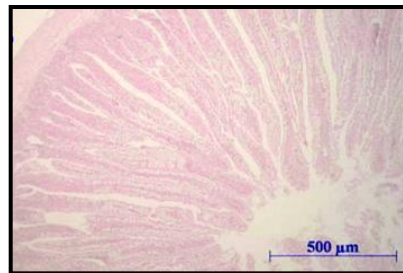
*Lactobacillus
fermentum*



*Escherichia
coli*



*Complex
microbiota*

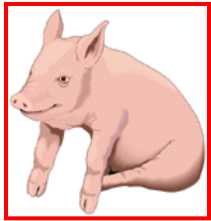


Decreasing villus lengths

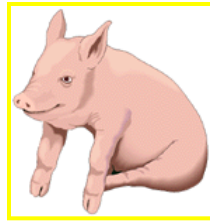


iTRAQ-labeling

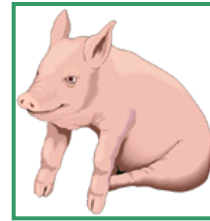
Peptides from **4** different samples are labelled with unique **mass tags**



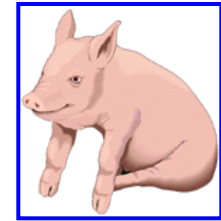
Reference - 114



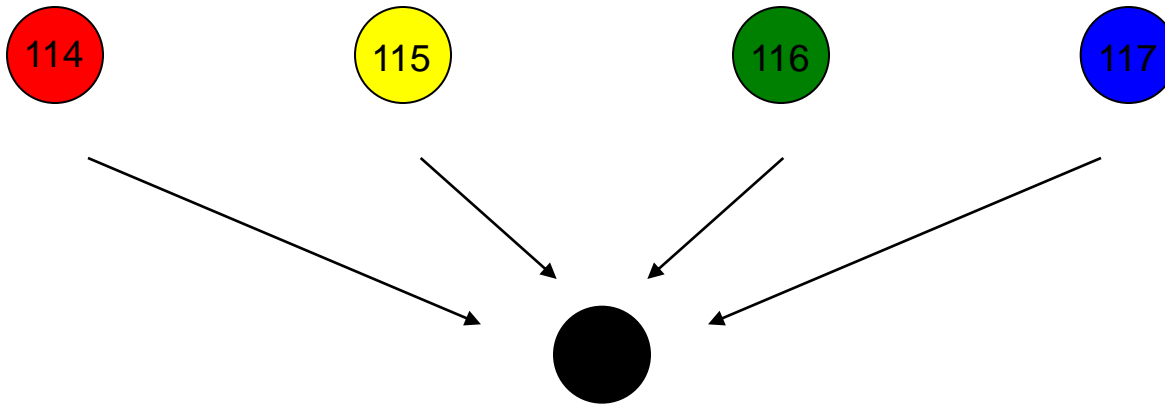
GF - 115



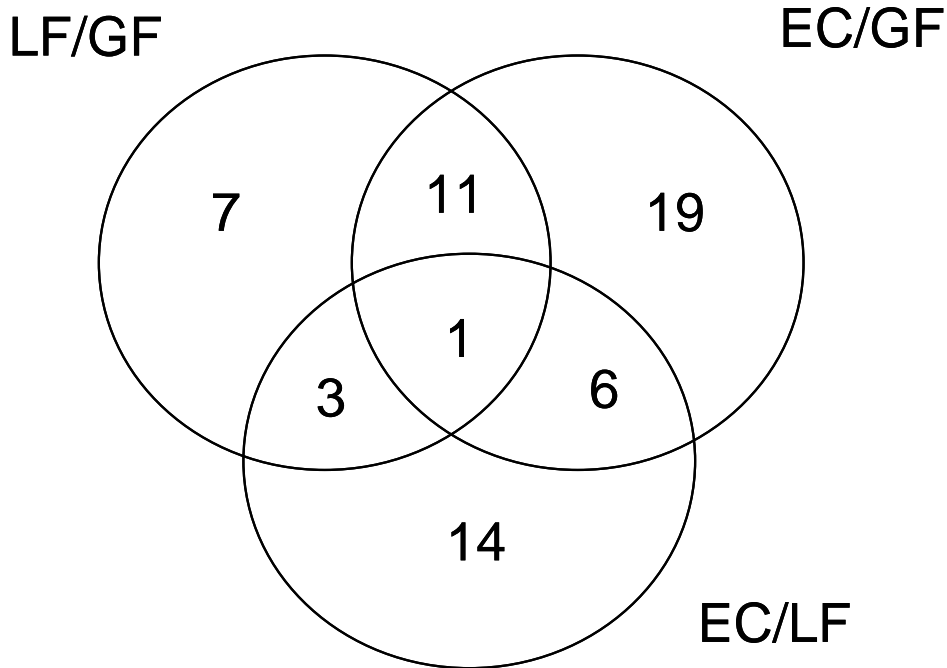
LF - 116



EC - 117



Regulated proteins



61 significantly regulated proteins

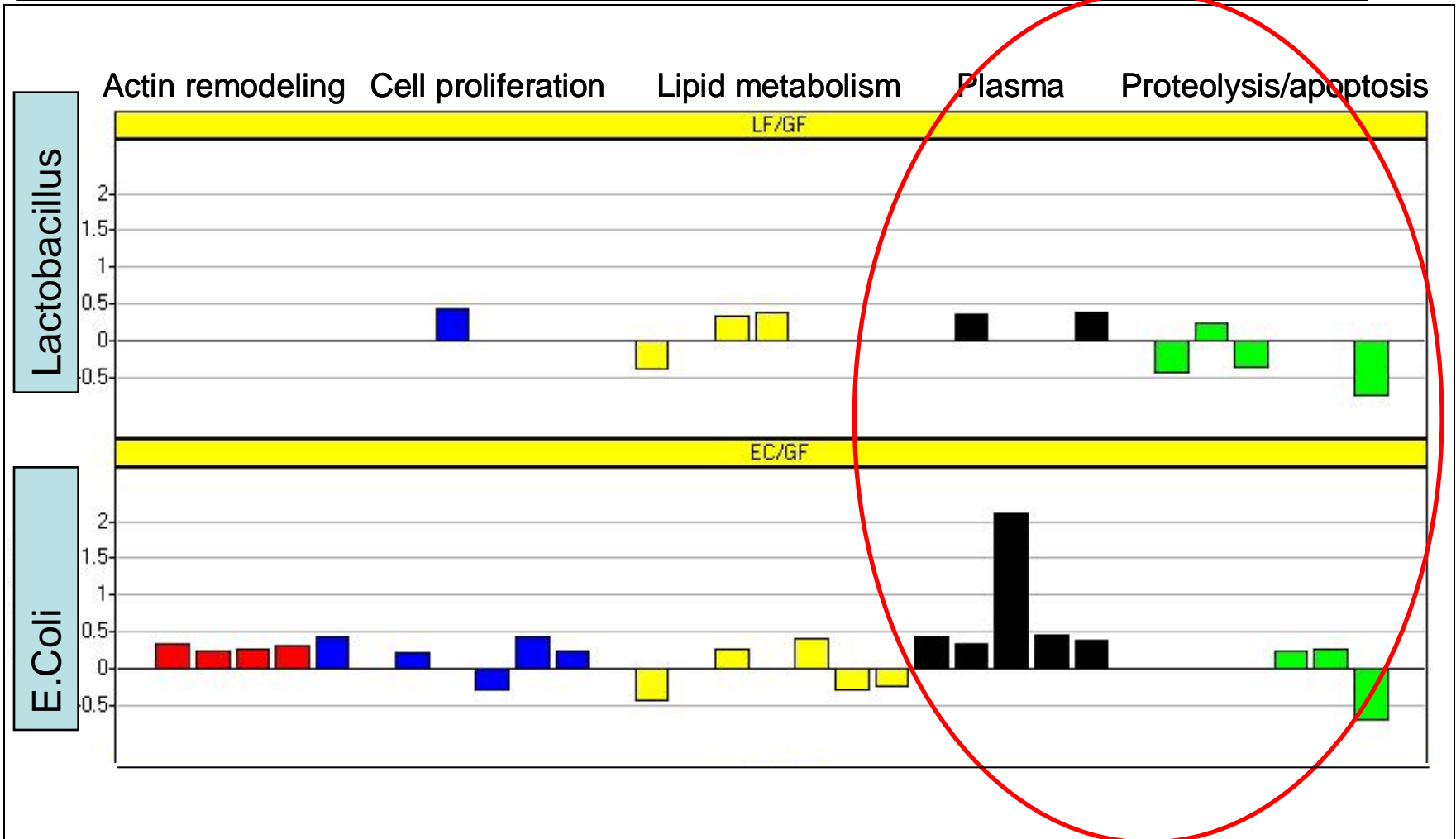
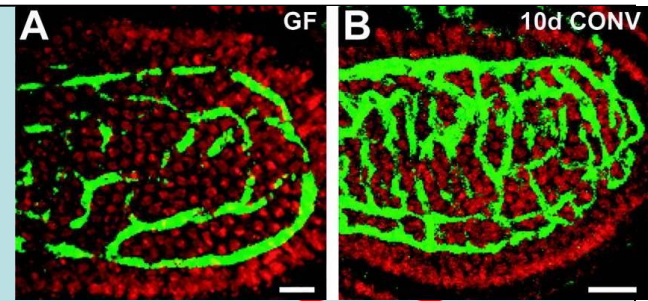
Contrasts:

- LF/GF – least regulated proteins
- EC/GF – most regulated proteins

AGREEMENT WITH MORPHOLOGY OBSERVATIONS

Colonization stimulates the formation of blood vessels in villi

Stappenbeck, et al. (2002)
Proc. Natl. Acad. Sci. USA 99, 15451-15455



Gnotobiotic pig - results

Both *L. fermentum* and *E. coli*:

- Affected lipid metabolism
- Stimulated the formation of new blood vessels

E. Coli **selectively** stimulated:

- enterocyte cell migration (actin re-modeling)
- cell proliferation

Conclusion: Yes, different bacteria does have different impacts on the tissue development

(Danielsen et al, 2007, Journal of Proteome Research)

Gut microbiota and obesity



firmicute

bacteroides



Infectobesity



Germ-free + "obese" microbiota

Germ-free + "lean" microbiota



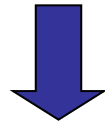
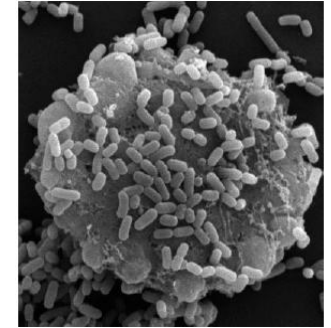
Mice that inherit obese-biota gain more weight!!!



(Turnbaugh et al. (2006) *Nature* 444, 1027-131)

Host-bacterial interaction

- 10 x bacteria than human cells
- 500-1500 bacterial species



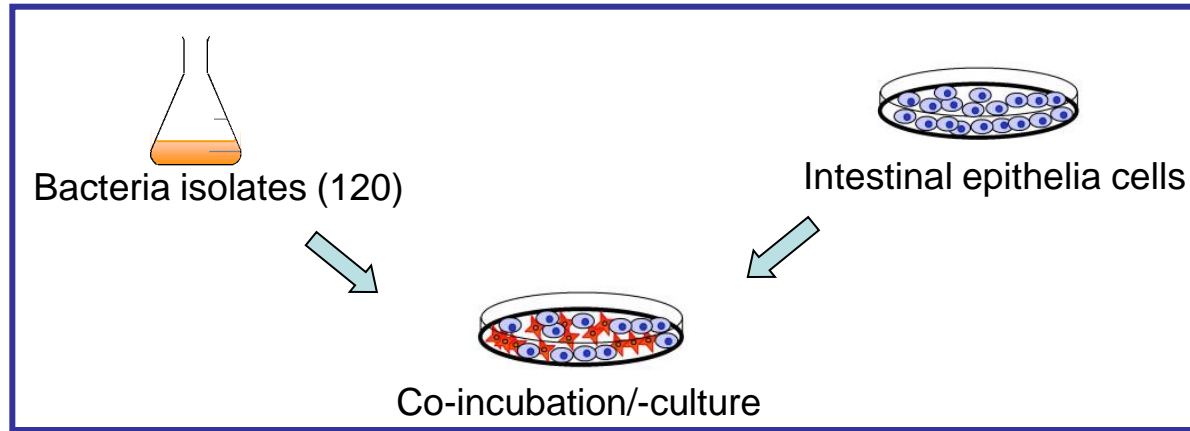
Ultra-complex functional genomics!



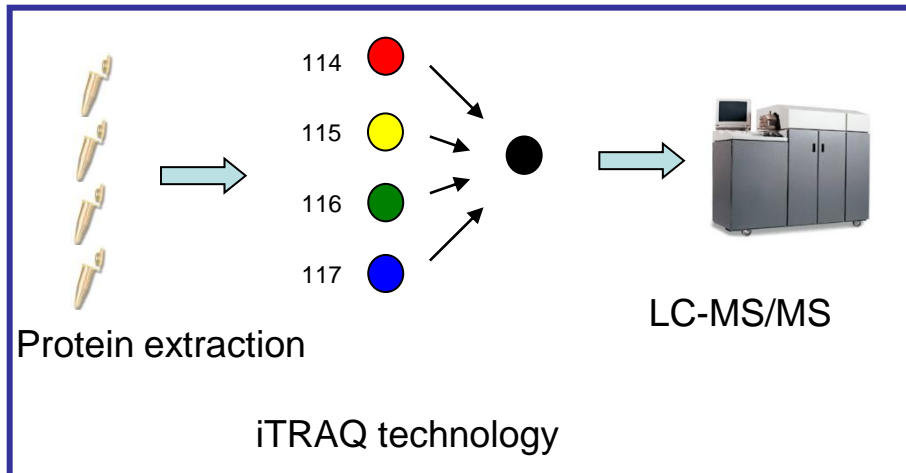
Infectobesity:

- a co-culture screening approach

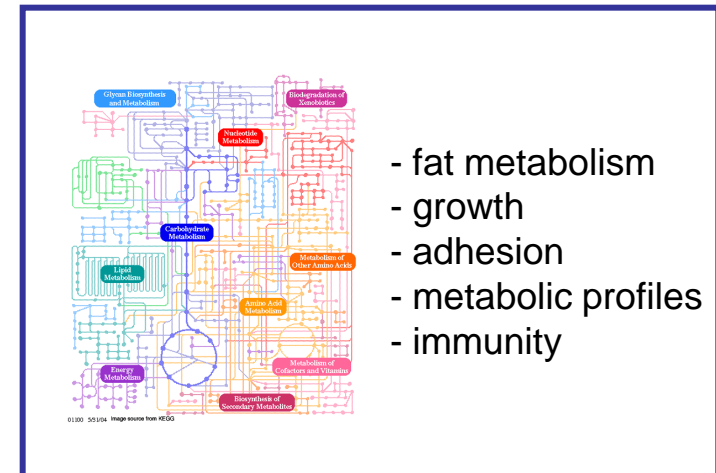
Co-cultures:



Proteomics:

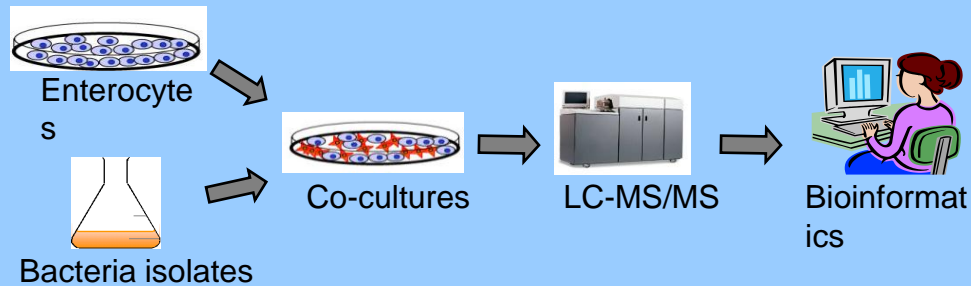


Pathway analysis:



co-cultures of intestinal cells and bacteria

- Human fetal small intestinal cells (FHs74Int) was co-incubated with *Bacteroides fragilis* og *Lactobacillum acidophilus*



- Results of proteomics from 2 hour incubation:

Glycolysis ↓

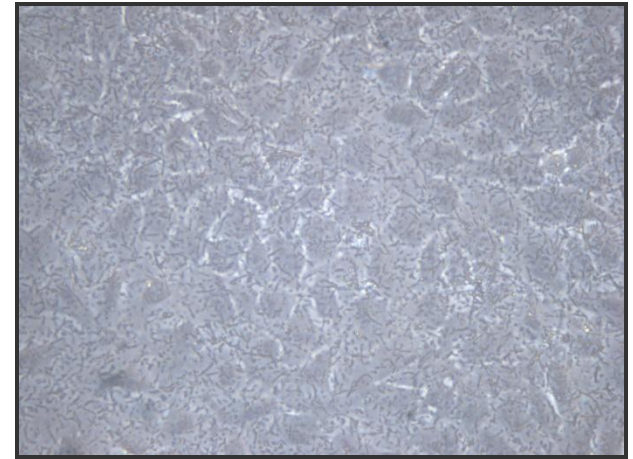
Detoxification ↑↓

Lipid metabolism ↑

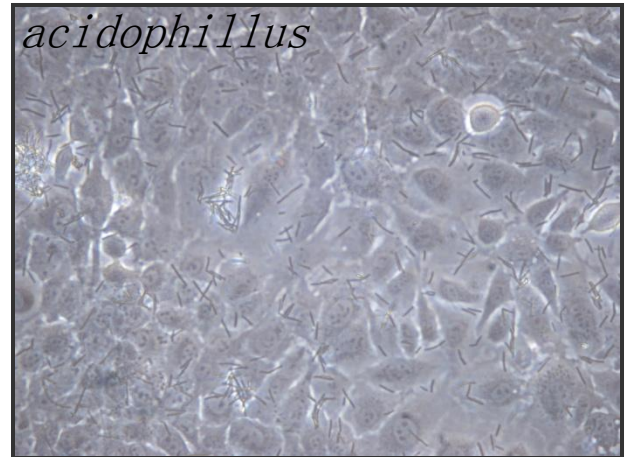
Ribosomal proteins ↓

Cytoskeleton proteins ↑

Bacteroides fragilis

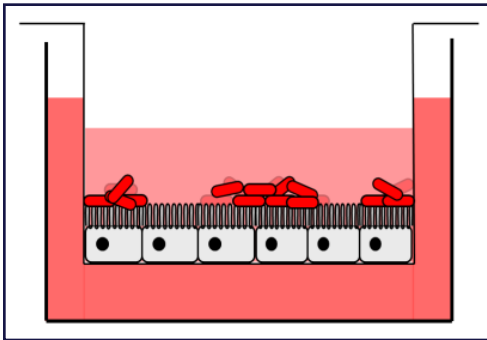
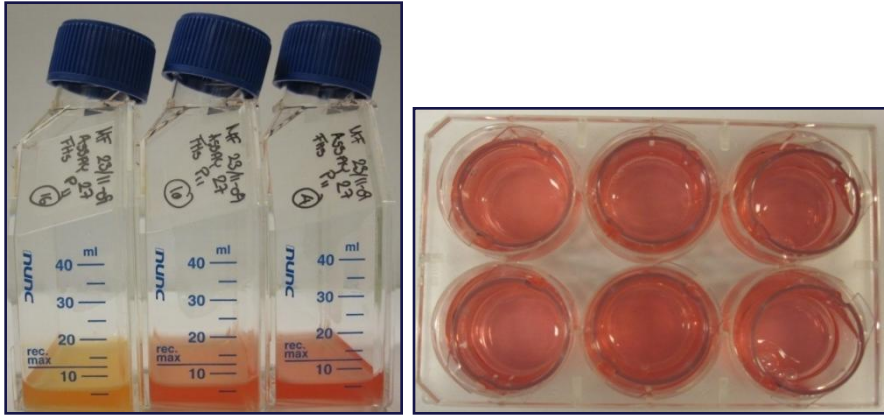


Lactobacillum acidophilus



co-cultures with Ecoli K-12

- Cell culture inserts



- FHs74Int: 0, 2, 8 and 24h

- **enzymes involved in CH metabolism**

- Glyceraldehyde-3-phosphatase ↓
- Fructose-biphosphate aldolase A ↓
- Triosephosphate isomerase ↓
- Alde-keto reductase family 1 member B10 ↓

- **Other proteins**

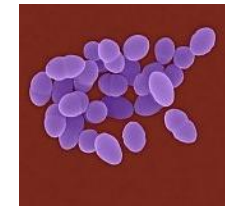
- ↓ proteins involved in energy metabolism
- ↓ inflammatory and pro-apoptotic proteins
- ↑ proteins involved in cell growth

Next step: SRM-based methods for screening selected metabolism markers



Mastitis in dairy cows

- Inflammation of the udder and mammary gland
- Several pathogens can cause mastitis
- The major problem in dairy cattle industry
- Affects 10% of lactating cows
- Major genetic influence on host response (QTLs)
- A major welfare and economic issue



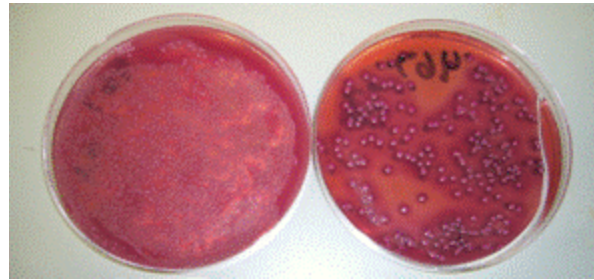
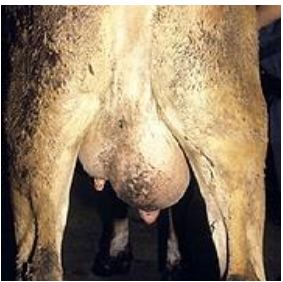
Gram +



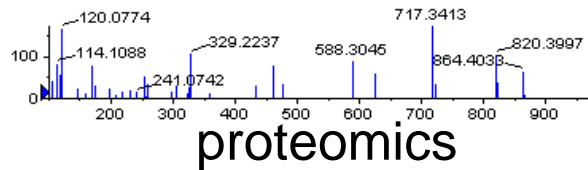
Gram -

Diagnosis of mastitis

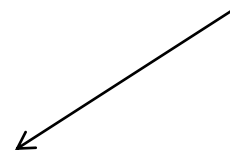
Current methods:



The aim :



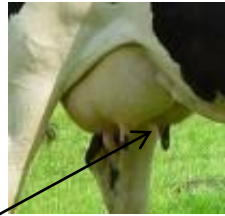
early biomarkers



On-line detection in milking robots

iTRAQ-analyses of milk proteins after LPS challenge

3 X



LPS (Ecoli
endotoxin)

MILK

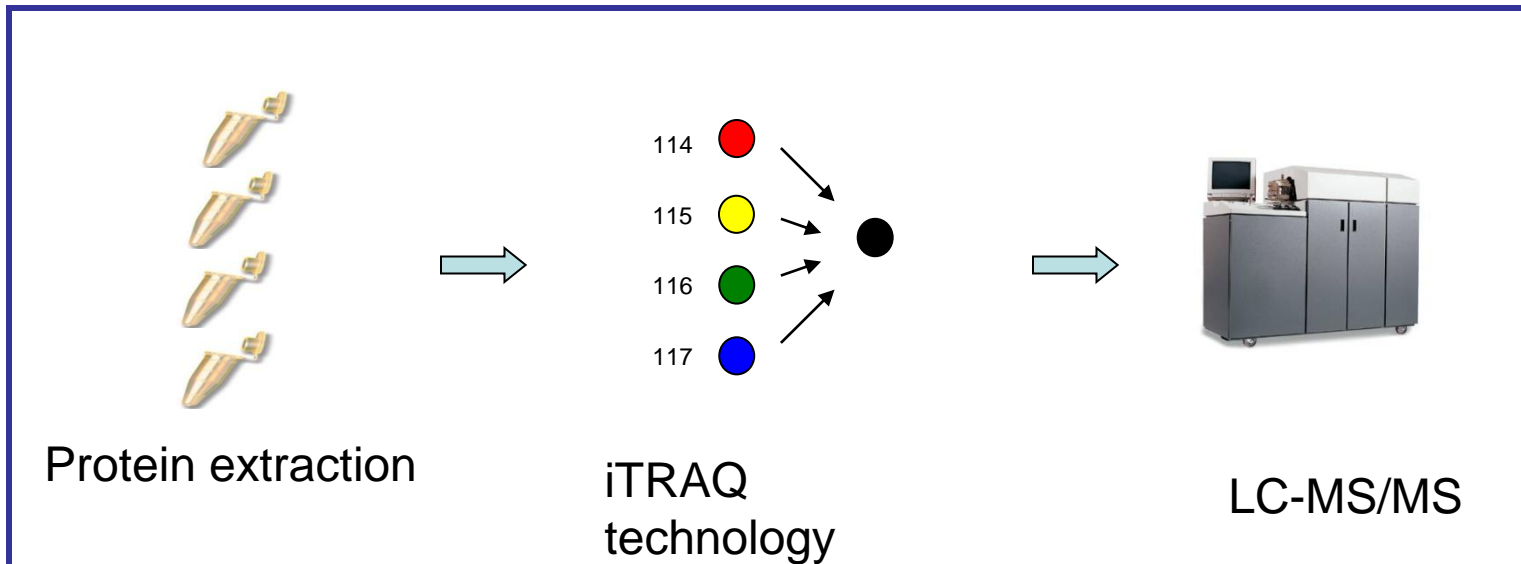


Reference – time= -3

Time =0

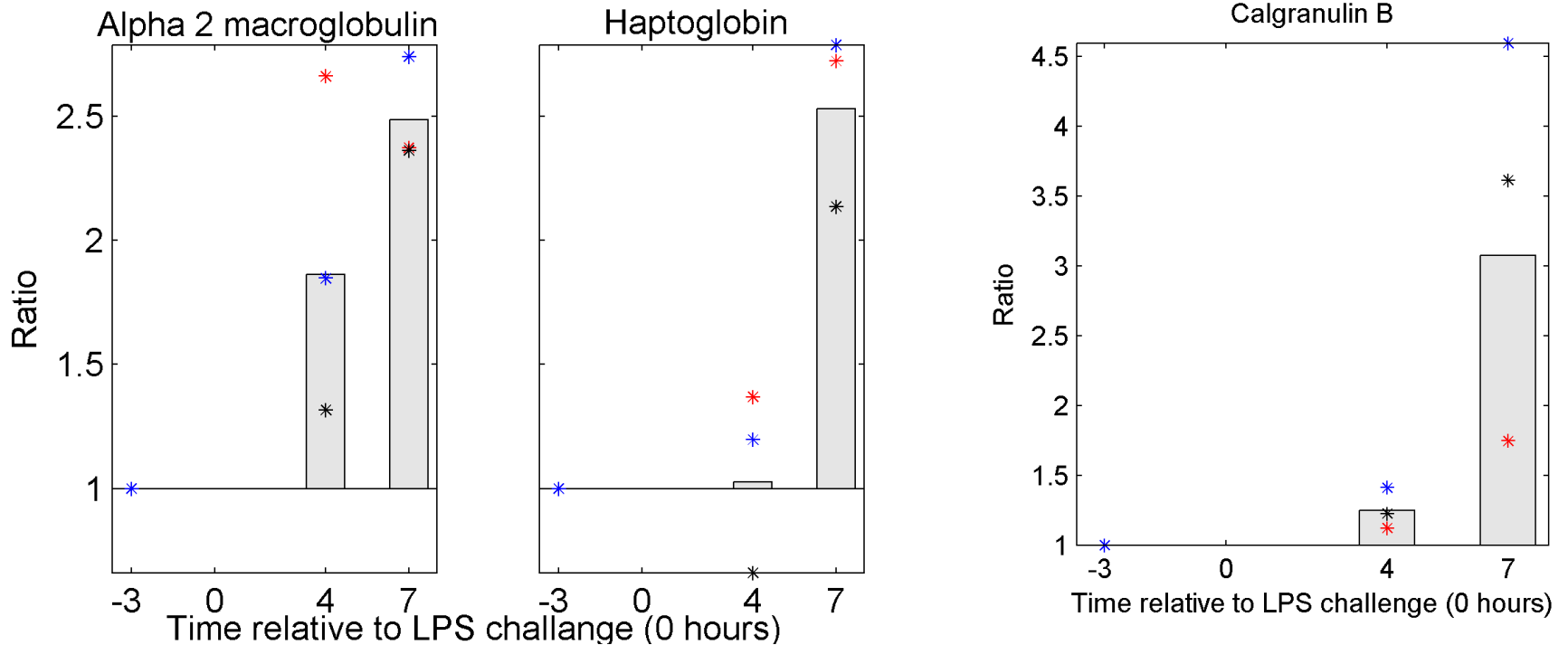
Time=4

Time=7



Host responses to LPS, detected in milk from 3 individual cows

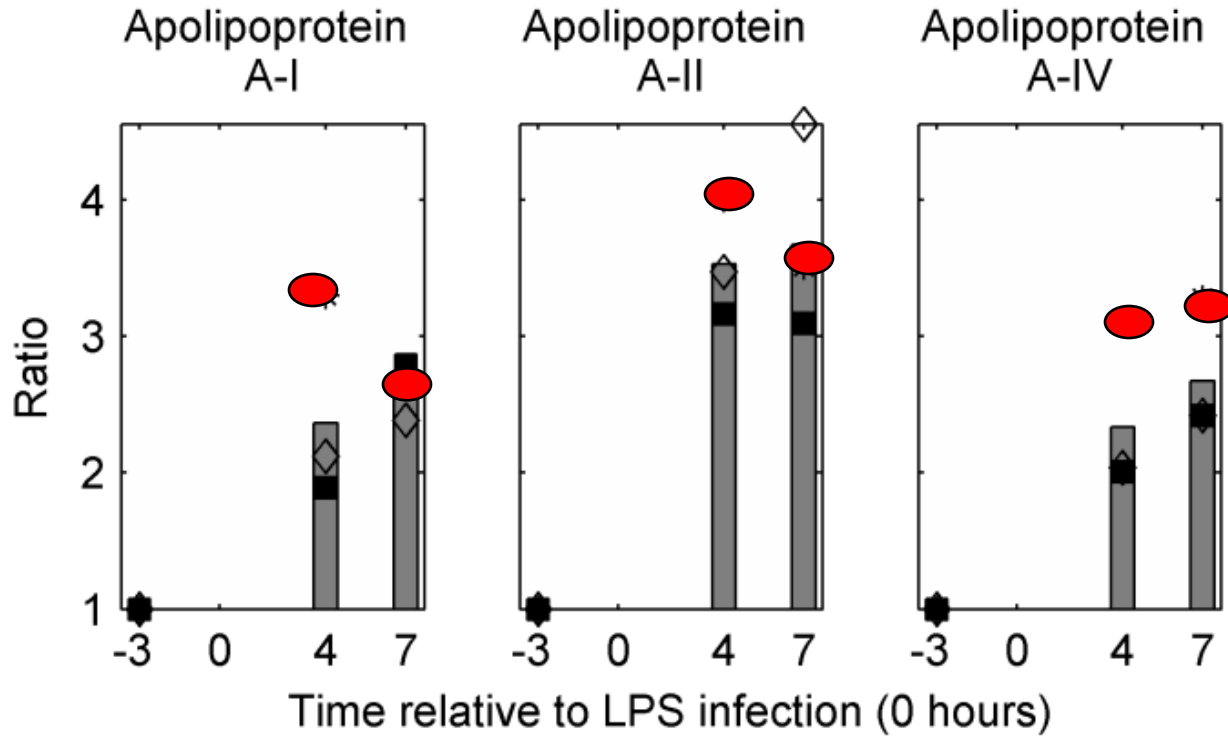
(Danielsen et al., Proteomics, 2010)



Acute phase response

Calgranulin B

Regulation of Apo (A) after LPS challenge



From shot-gun to targeted proteomics

Shot-gun proteomics



- Discovery-based
- Relative quant
- High abundant proteins
- ~100 most abundant milk proteins detected

Targeted proteomics



- Hypothesis-based
- Absolute quant
- Low abundant proteins

Detection range: 50 – 10^6 copies/cell

(Picotti et al., 2009, Cell 138, 795-806)

Building Peptide Atlas of pig and cattle

- Collaboration with Institute for Systems Biology, Seattle
- (Eric Deutch, Therry Farrah & co)
- **Bovine atlas;**
- Milk , mammary gland, cultured cells, Immune cells,
- >2000 proteins
- > 20 000 peptides
- **Porcine Atlas:**
- > 20 different tissues
- > 7000 proteins
- > 50 000 peptides



Marius Codrea

Stine Lønnerup

Ensembl Protein [ENSBTAP00000012939](#)
 Entrez Gene Symbol [CXCL5](#)
 Entrez GeneID [281735](#)
 IPI [IPI00691126](#)
 RefSeq [NP_776725](#)
 UniGene [Bt.7165](#)
 UniProt [P80221](#)
 UniProt/TrEMBL [Q9TR91](#)
 Distinct Peptides 2
 Total Observations 5

External Links

Sequence Motifs

Sequence

MRLISSRAAR VSGPSGSLCA LLALLLTPP GPLASAGPVA AVVRELRCVC LITTPGIHPK TVSDLQVIAA
GPQCSKVEVI ATLNKGREVC LDPEAPLIKK IVQKILDSGK NN

Protein Coverage = 25% (31.4% of likely observable sequence)

[show column descriptions](#)

Distinct Observed Peptides (2)

Peptide Accession	Pre AA	Peptide Sequence	Fol AA	Suitability Score	Best Prob	N Obs	EOS	RHS	N Protein Mappings	N Genome Locations	sample_ids	is_subpeptide_of
PAp01177434	R	EVCLDPEAPLIK	K	0.85	0.969	3	30.19		2	1	2317,2323	
PAp01182599	K	TVSDLQVIAAGPQCSK	V	0.83	1.000	2	25.11		3	1	2323,2378	

Proteotypic peptides



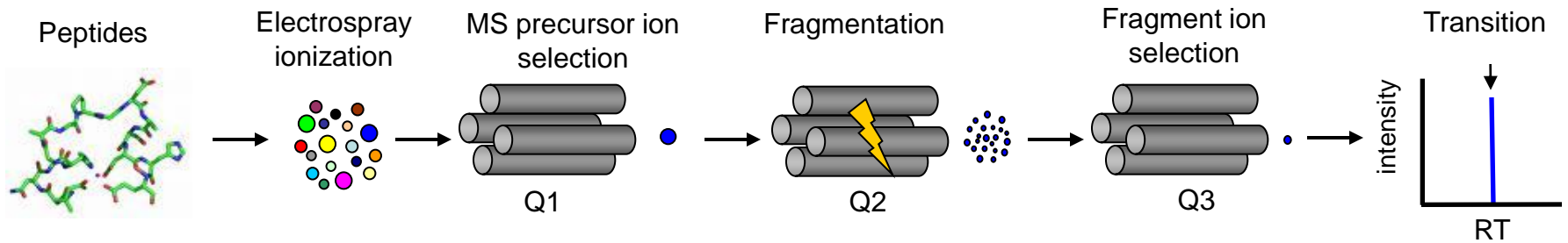
[View peptides and related proteins as a network with Cytoscape]

Predicted Highly Observable Peptides

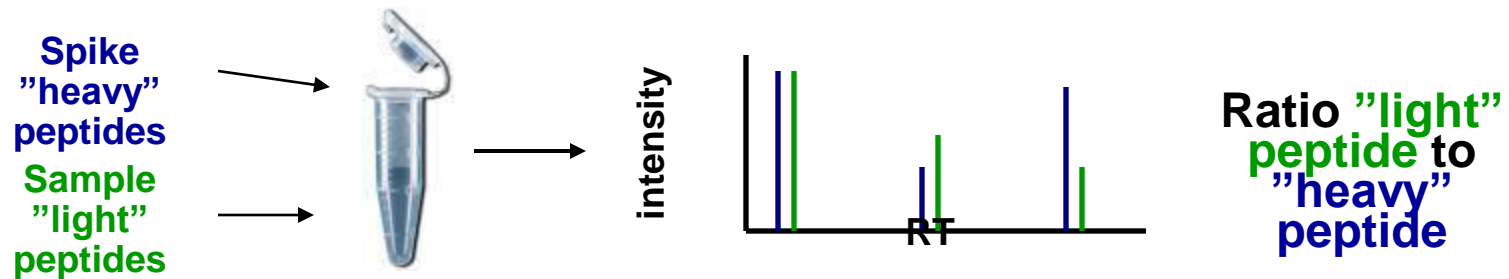
Peptide Accession	Pre AA	Peptide Sequence	Fol AA	Suitability Score	Detectability Predictor Score	PeptideSieve Score
	K	TVSDLQVIAAGPQCSK	V	0.83	0.73	0.93
	R	EVCLDPEAPLIK	K	0.58	0.48	0.67

Selected reaction monitoring (SRM)

Identification



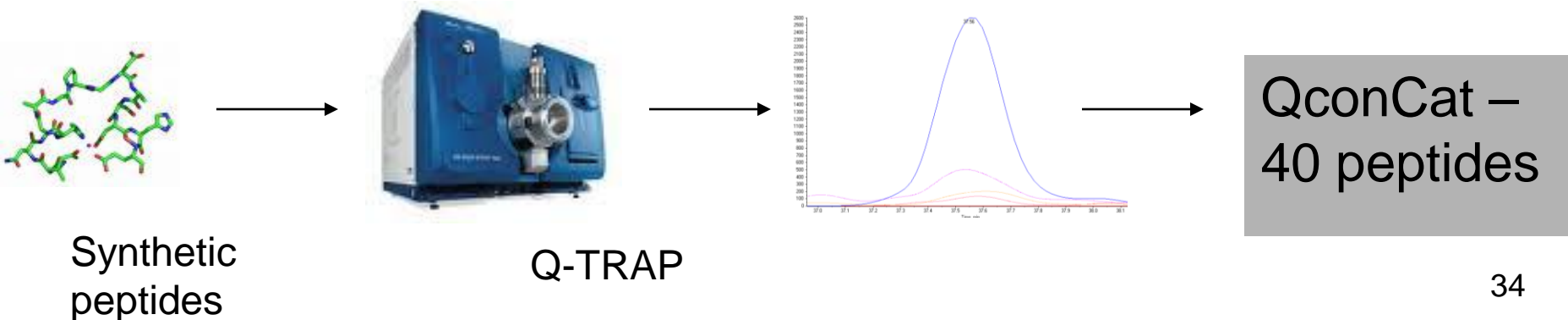
Quantification



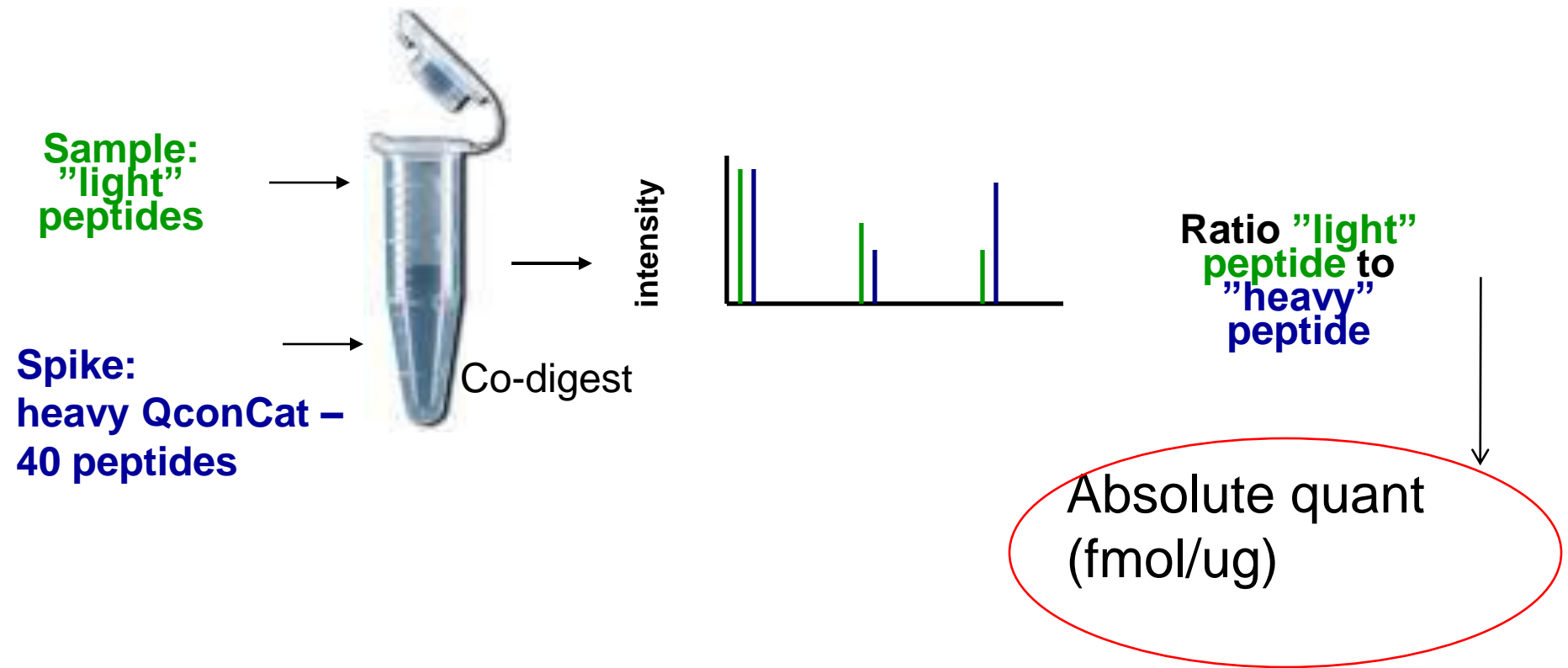
Transitions for 20 target-proteins have been optimised.

High-abundant proteins	Number of peptides detected
Lactoferrin	4 / 4
Alpha-1-antitrypsin	4 / 4
Alpha-2-macroglobulin	4 / 4
LGALS1	3 / 4
LGALS3	3 / 4
SAA3	3 / 3
S100A12 - Calgranulin C	4 / 4
Calgranulin B	4 / 4
Cathepsin C	3 / 4
Haptoglobin	4 / 4

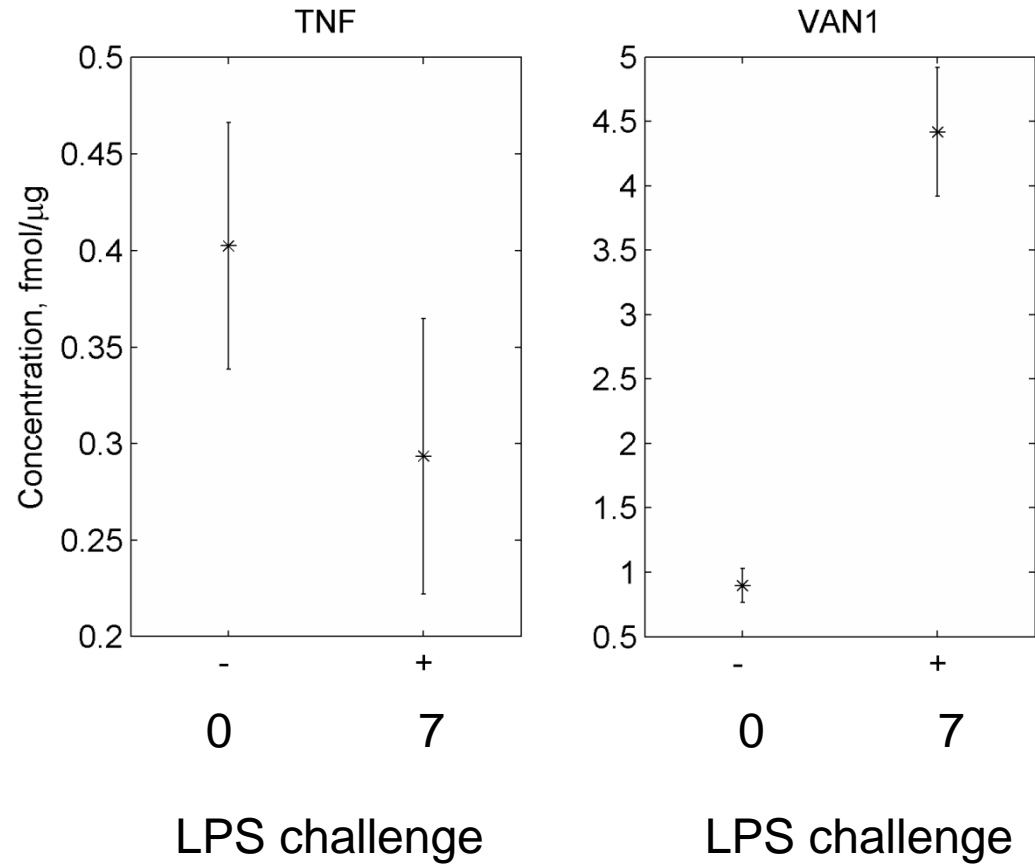
Low-abundant proteins	Number of peptides detected
Vanin 1	4 / 4
Tumor Necrosis Factor α (TNF- α)	4 / 4
CD14	3 / 4
Interleukin 6	3 / 4
Interleukin 8	3 / 4
Interleukin 1 RN	3 / 4
Interleukin 1 β	2 / 4
Interleukin 6 receptor	0 / 4
CXCL1	2 / 4
CXCL3	1 / 2

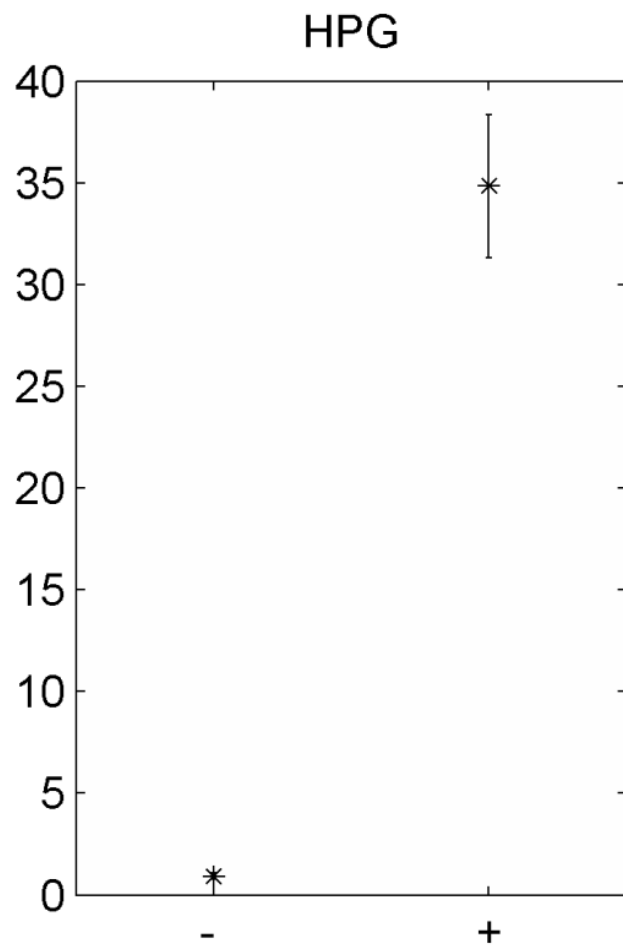
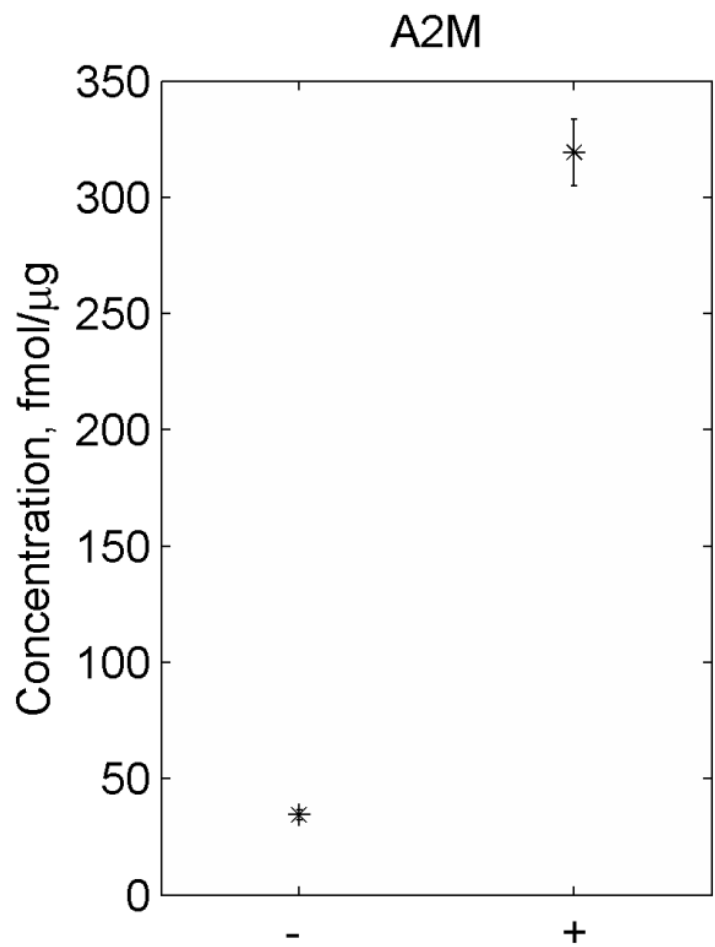


Quantification by heavy QconCat peptide spiking and SRM.



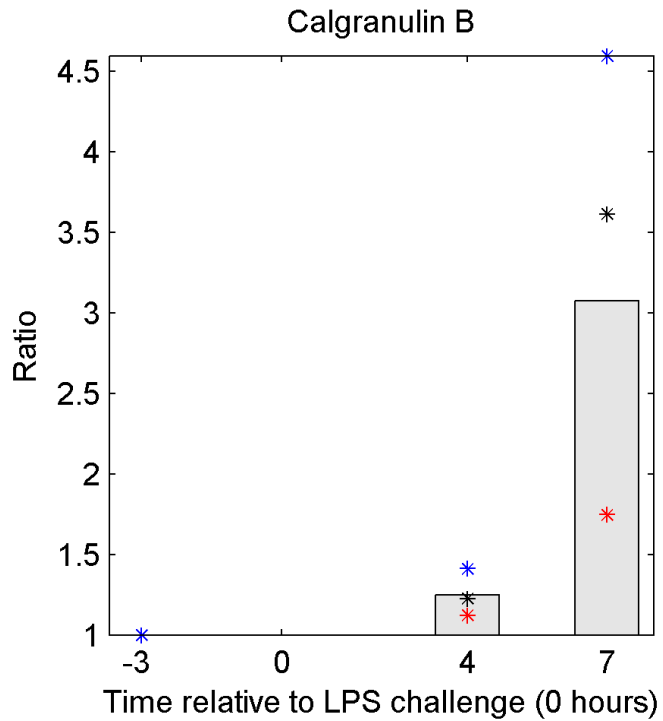
Milk samples spiked with (H)QconCat peptides



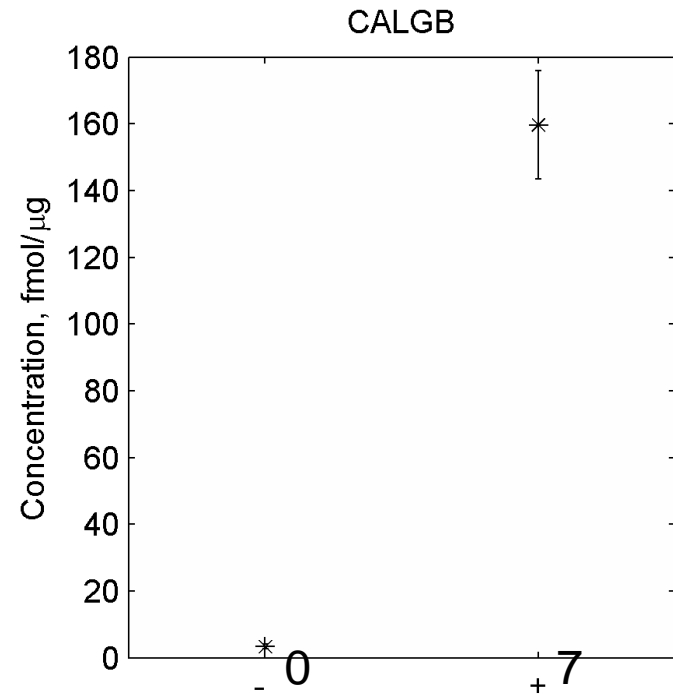


LPS challenge

Relative versus Absolute quant data



iTRAQ



Spiked with 5 fmol(H)QconCat peptides

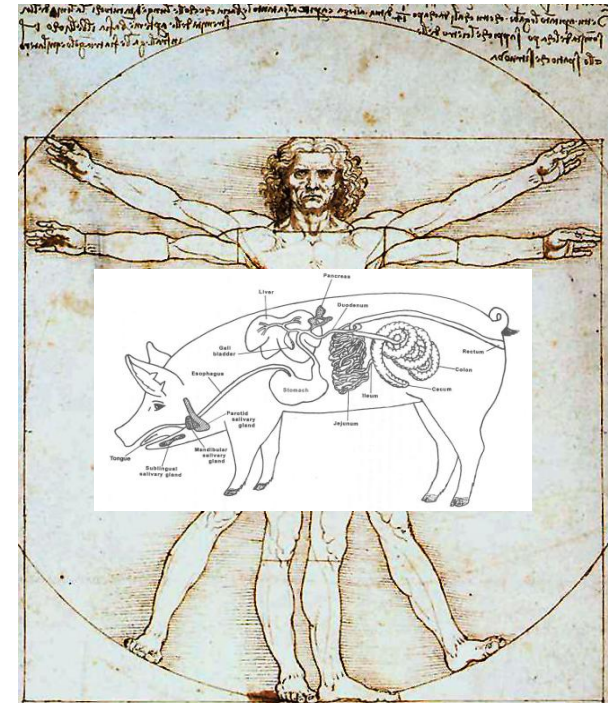
“We must measure what can be measured,
and make measurable what cannot be
measured.”

Galileo Galilei 1610

Farm animals provide important biological variation for systems biology

Solving the problems of farm industry

Model organisms for human biology



Thanks to

Stine Lønnerup Marius Codrea

Colleagues at Århus University

- Dept. of Animal Health
- Dept. of Genetics

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- Andrew Van Kessel (University of Saskatchewan)
- Richard Siggers (University of Copenhagen)
- Rob Moritz, Eric Deutsch, Ulrike Kusebau, Terry Farrah (Institute of Systems Biology, Seattle)
- Ruedi Aebersold (University of Zurich)
- Rob Beynon (University of Liverpool)

Funding and Industrial partners

- Danish Cattle Industry
- Danish Pig Industry
- Ministry of Food, Agriculture and Fisheries
- Danish National Research Foundation
- EU-funding



Marianne Danielsen

Marlene Fredgård