

ANIMAL PROTEOMICS: IPG-DALT and more...

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ANIMAL PROTEOMICS:

- ❖ **animal science:**
animal medicine (biomarker search)
- ❖ **food industry:**
animal derived products
- ❖ **animal models:**
study of diseases / expositions
- ❖ ...

ANIMAL PROTEOMICS:

- ❖ **animal science:**
animal medicine (biomarker search)
- ❖ **food industry:**
animal derived products (**COST - EuFAP**)
- ❖ **animal models:**
study of diseases / expositions
- ❖ **technical modifications**

A VARIETY OF SPECIES

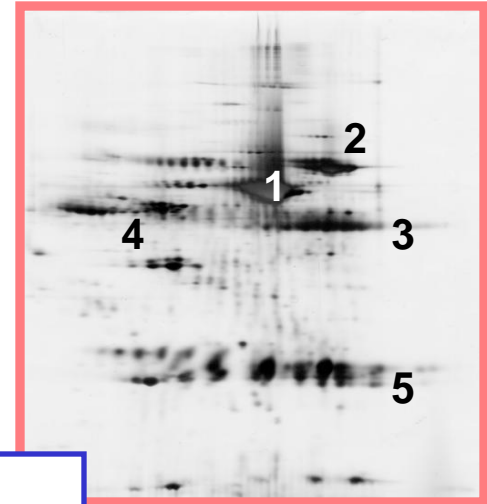
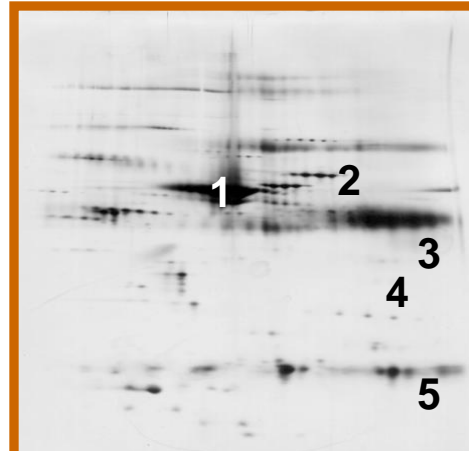
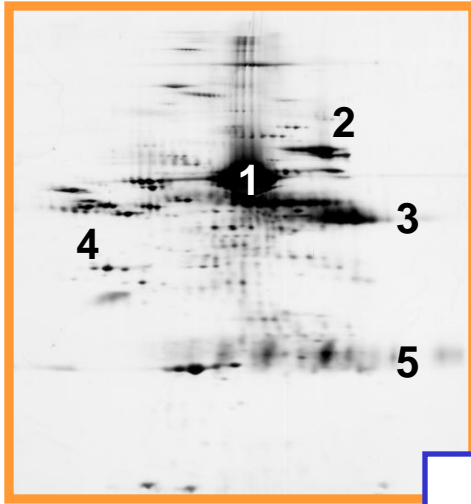
A VARIETY OF SPECIES

- differences in protein properties (pI, Mr...)
- differences in protein concentrations (health, disease)
- species specific proteins

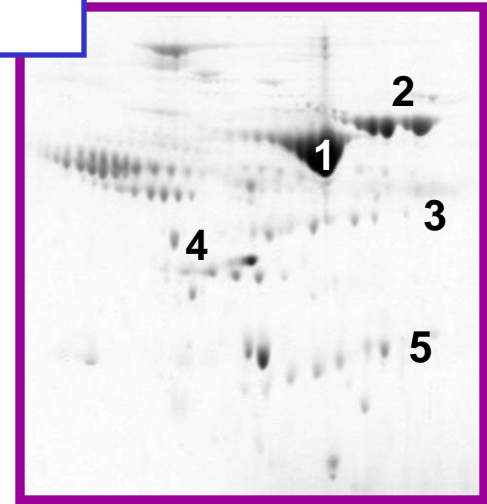
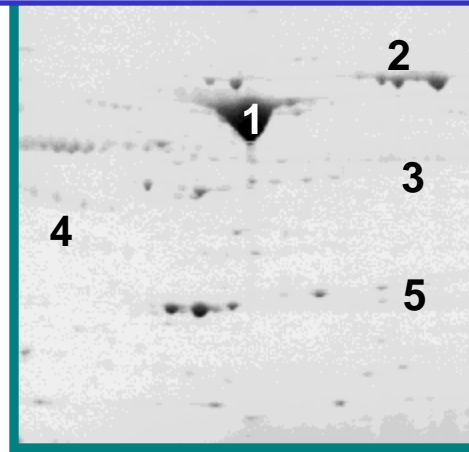
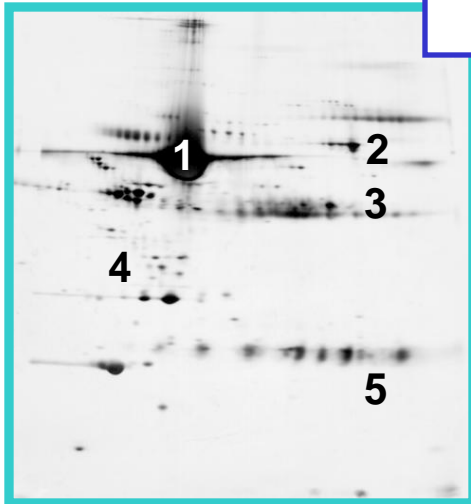
HORSE

COW

PIG



SERUM



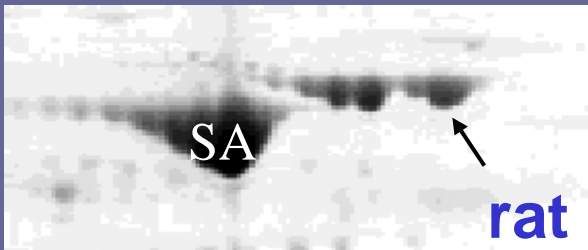
DOG

MOUSE

RAT

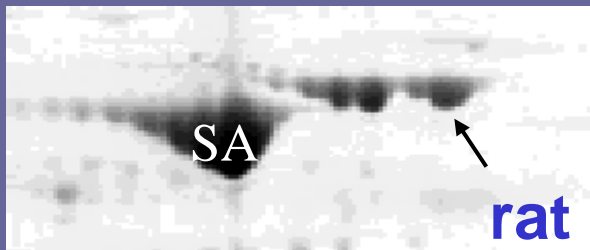
1- serum albumin; 2 – transferrin; 3 – Ig γ -chain; 4 – haptoglobin β -; 5 – Ig L-chain

transferrin



(and other species)

transferrin



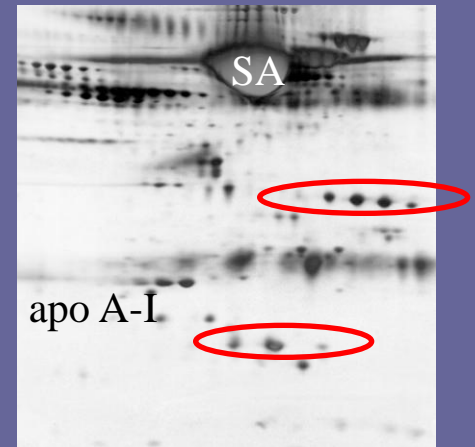
(and other species)

haptoglobin

COW

β -chain

α -chain



other species

α -chain



A VARIETY...

Positive Acute Phase Proteins (proteins up-regulated in inflammation)

	CRP	SAA	Hp	oroso	α_1 AT	other
human	++	+	+	+	+	
rat	±	x	+	+	+	α_1 MAP, SPI-3
dog	+	+	+	+	-	
cow			++			
pig	+	+	+	-	-	MAP (++)

x ... non existent

CRP cannot be detected in electrophoresis (except for rat)

EXAMPLES

- ❖ animal model of inflammation and shock (rat)
- ❖ transgenic models (mouse)
- ❖ physiological changes (cow)
- ❖ gammaglobulin disorders (dog)
- ❖ homologous proteins (fibrinogen)
- ❖ depletion

1. Animal model of inflammation and shock (rat)

**proteome of control
tissue / body fluid**

mechanism
of drug toxicity

pathological
process

infectious
process

**proteome
of tissue / biological fluid
after sham treatment**

**proteome of pathological
tissue / body fluid**

mechanism of
drug resistance

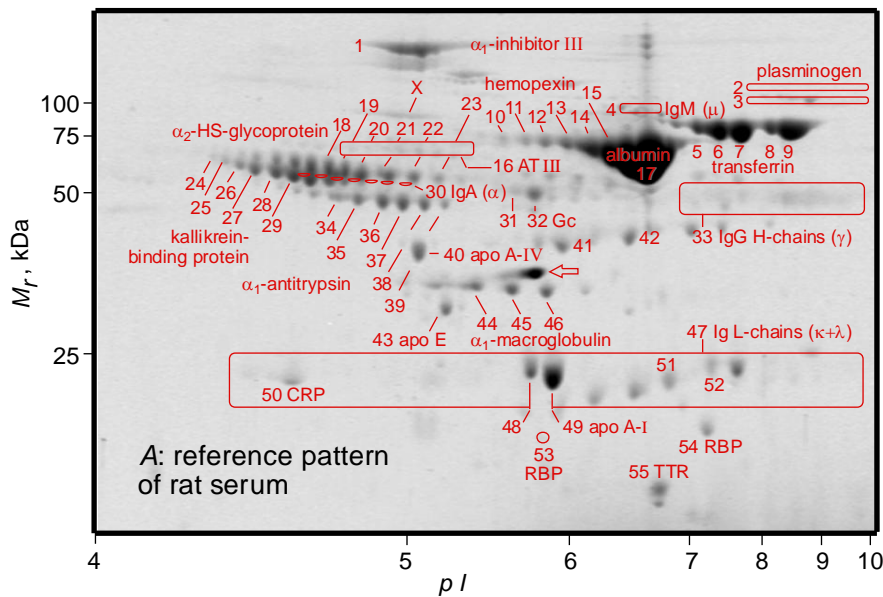
mechanism of
drug action

**proteome
of tissue / biological fluid
after therapeutic treatment**

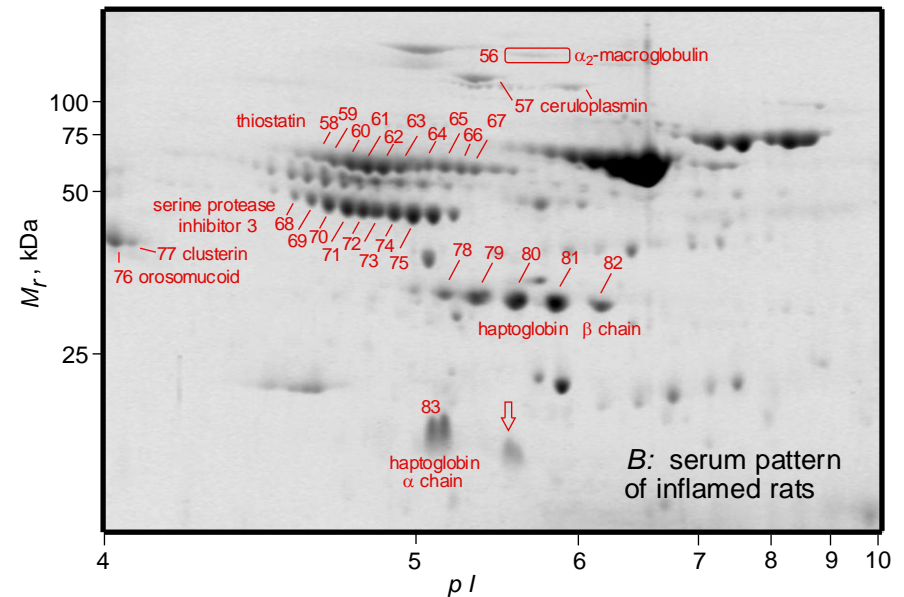


Serum protein pattern in inflammation

Rat serum protein patterns



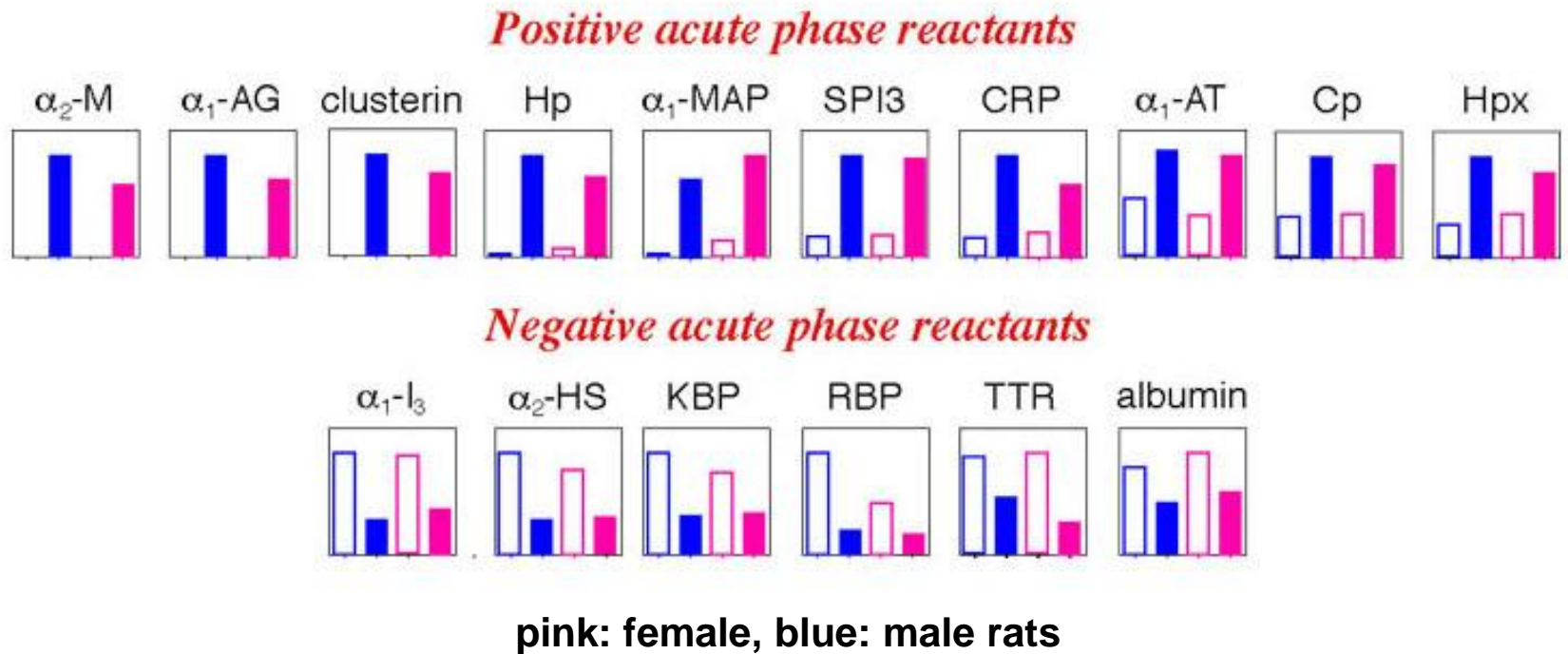
Serum (healthy)



Serum (inflamed, 48h)

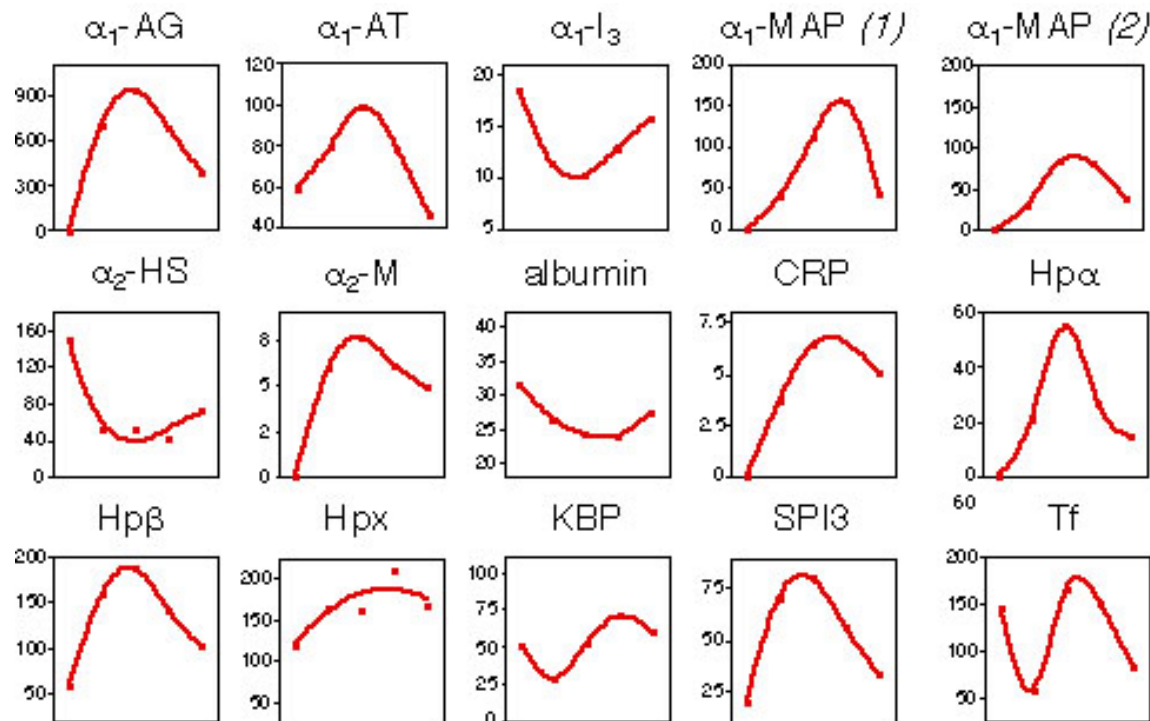
male Sprague-Dawley rats
i.m. turpentine

Protein concentration changes



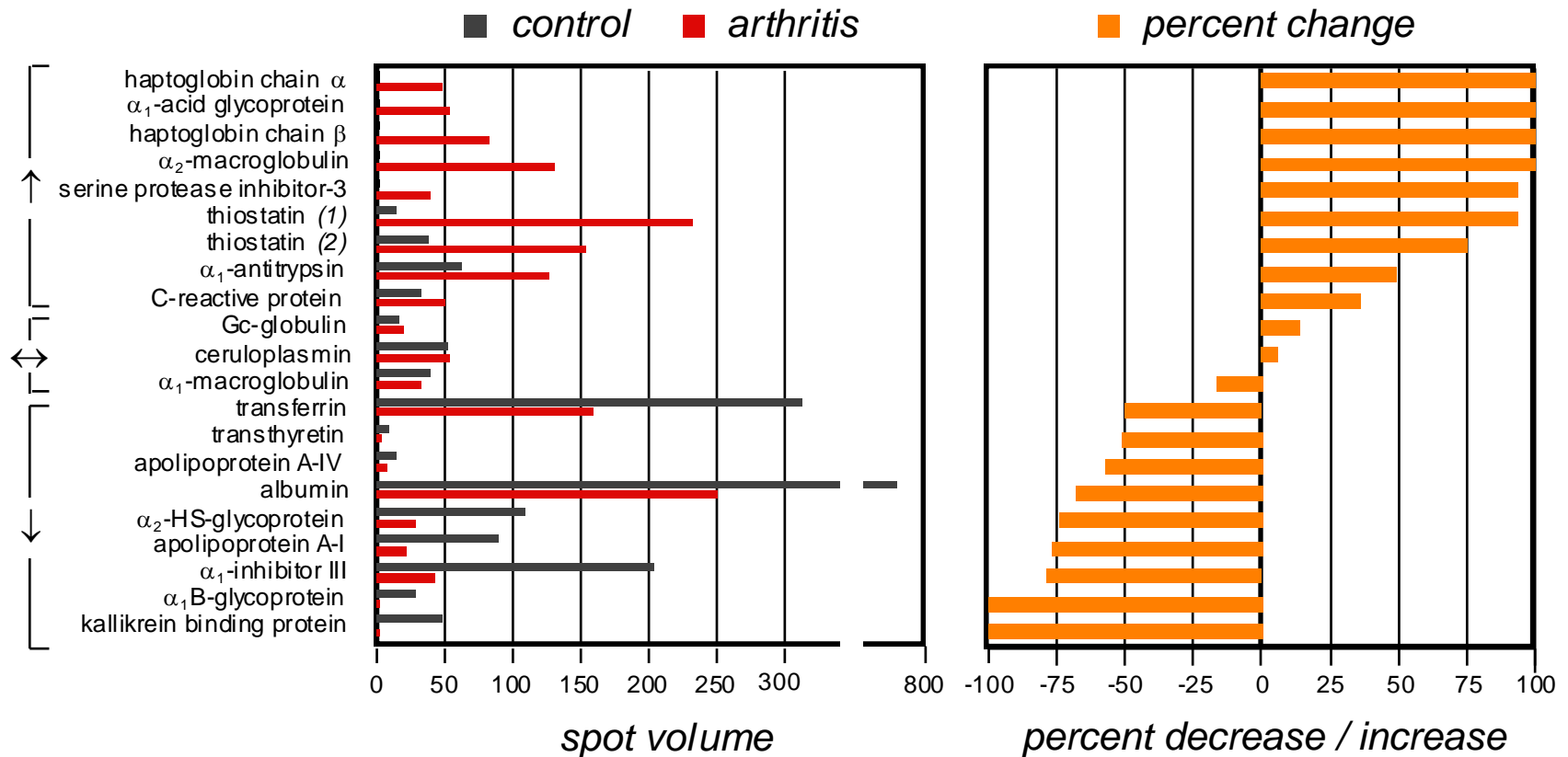
48 hours

Time-course

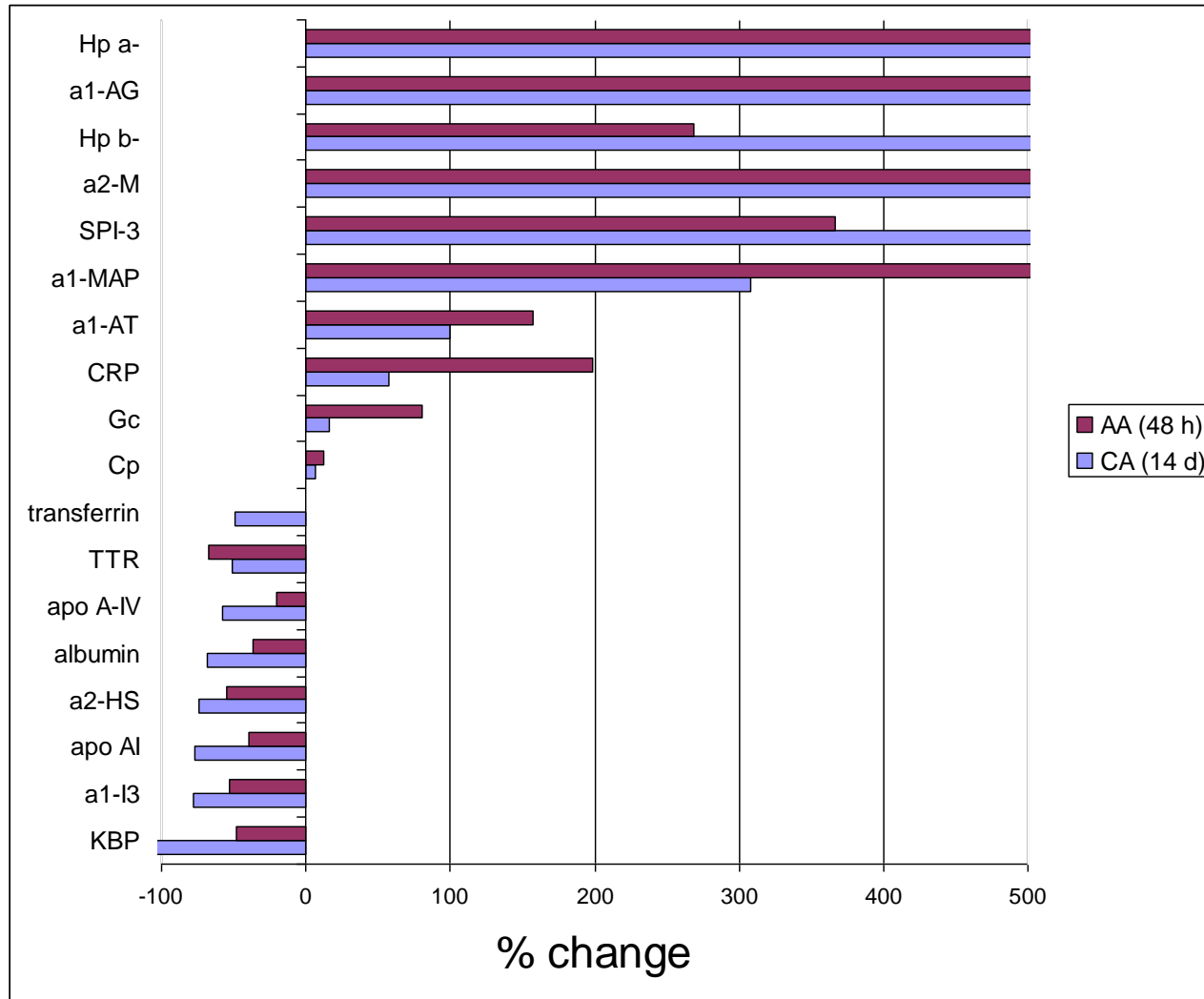


1 - 4 days

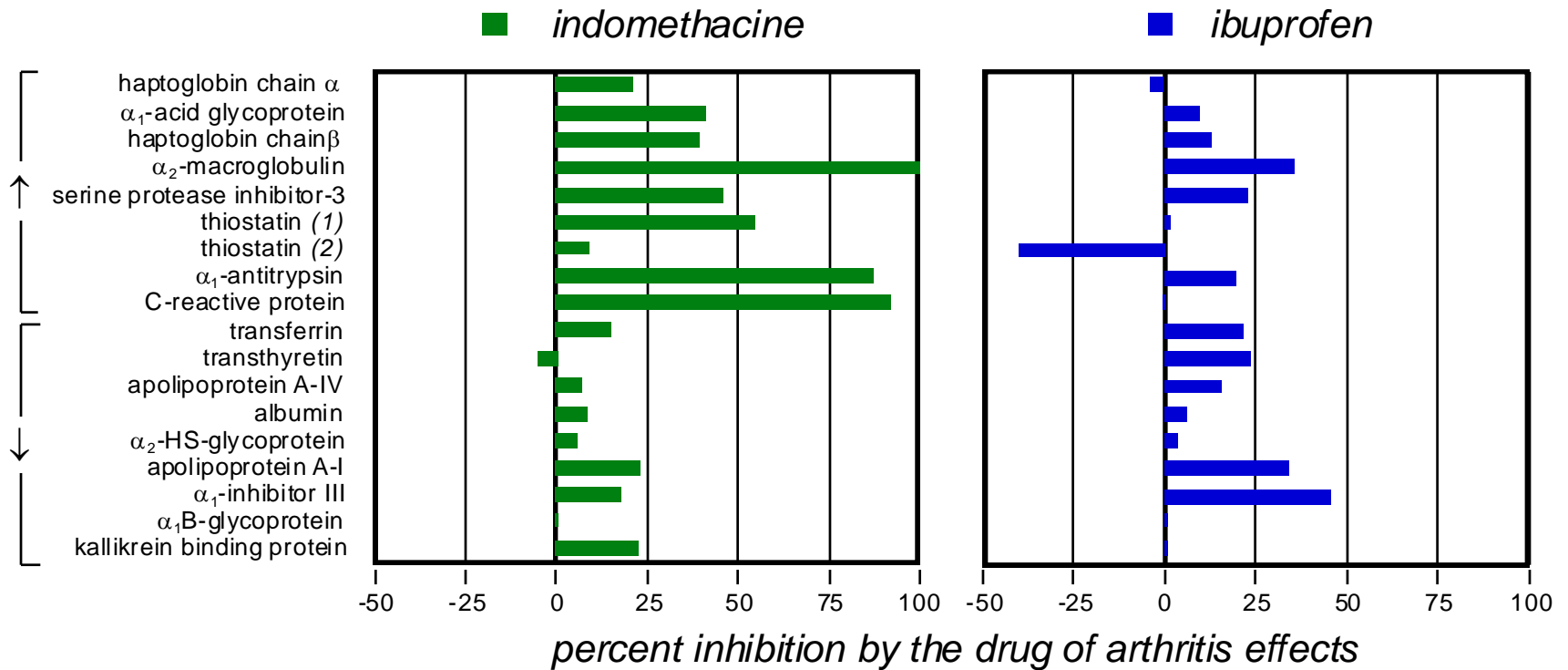
Effect of adjuvant arthritis on serum protein levels



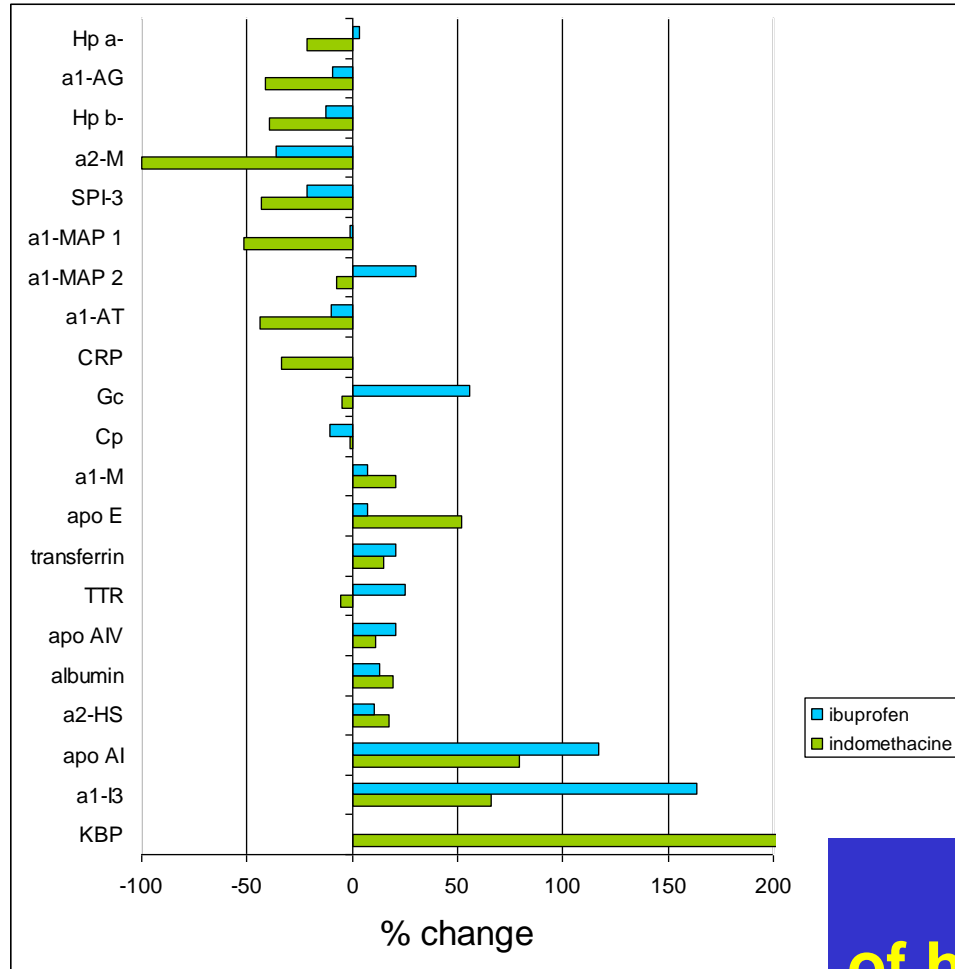
Differentially regulated proteins



Effect of NSAID treatment on serum protein levels in adjuvant arthritis



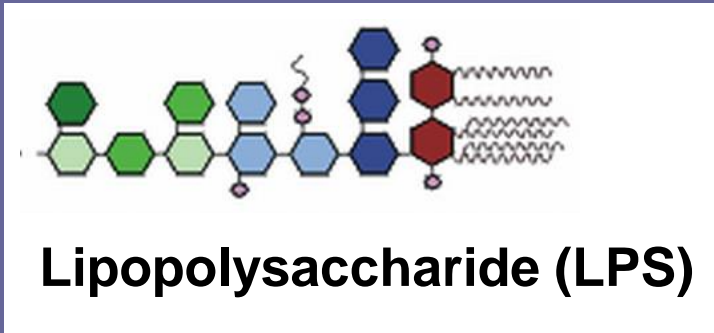
Effect of NSAID treatment per se on serum protein levels



Treatment of healthy animals

Liver proteome and endotoxic shock

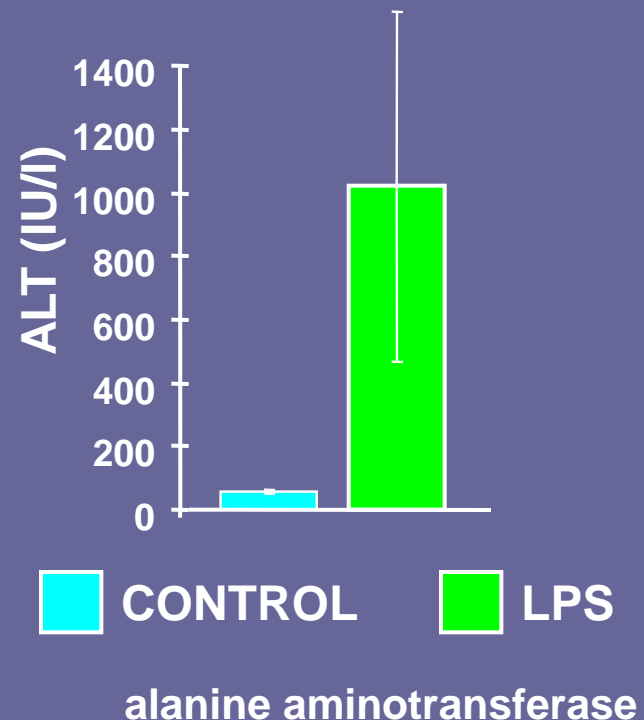
Experimental shock model:

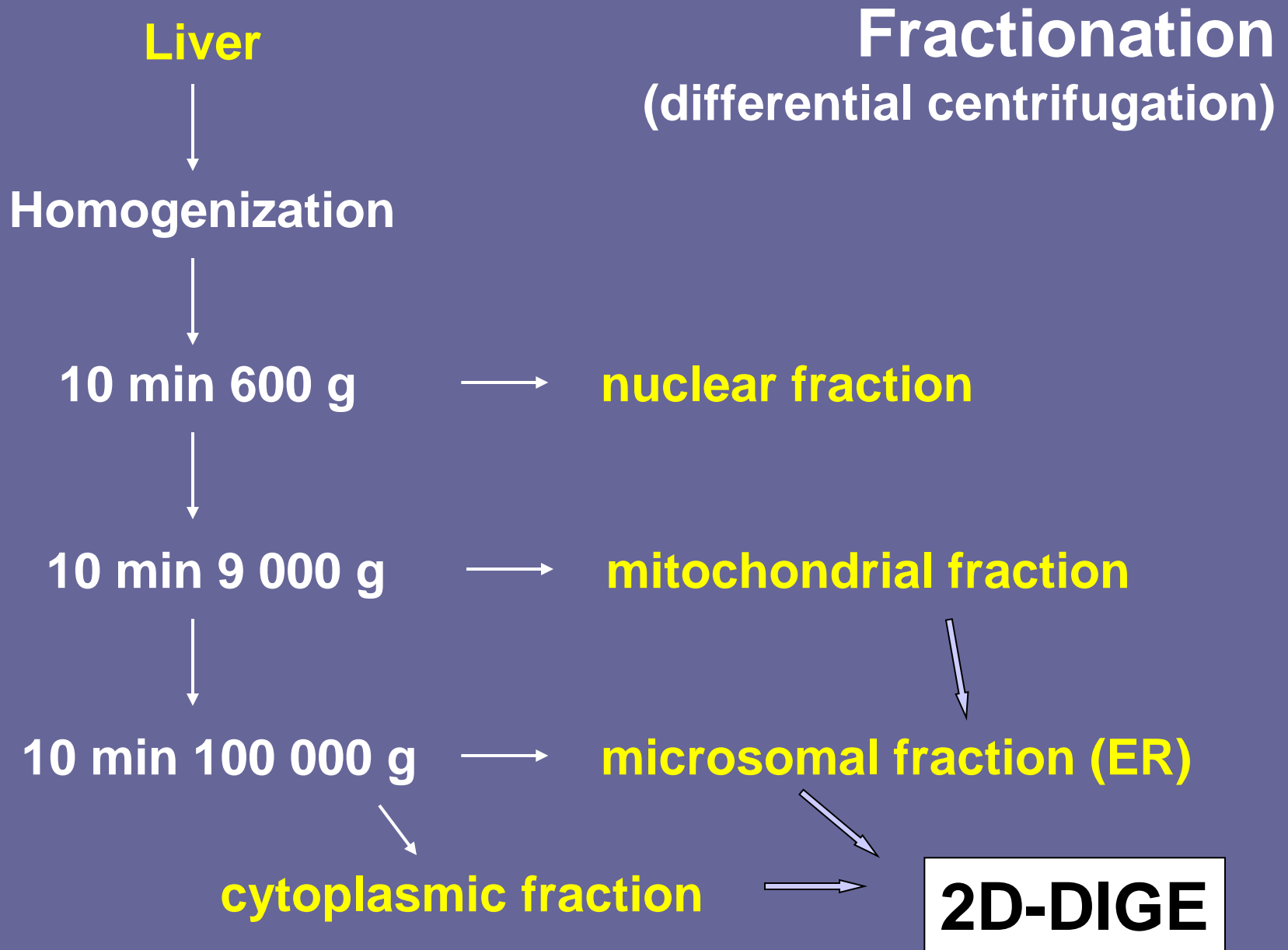


- male Sprague Dawley rats
- 8 mg/kg LPS i.p. / i.v.
- 16 hours after LPS challenge

Liver failure

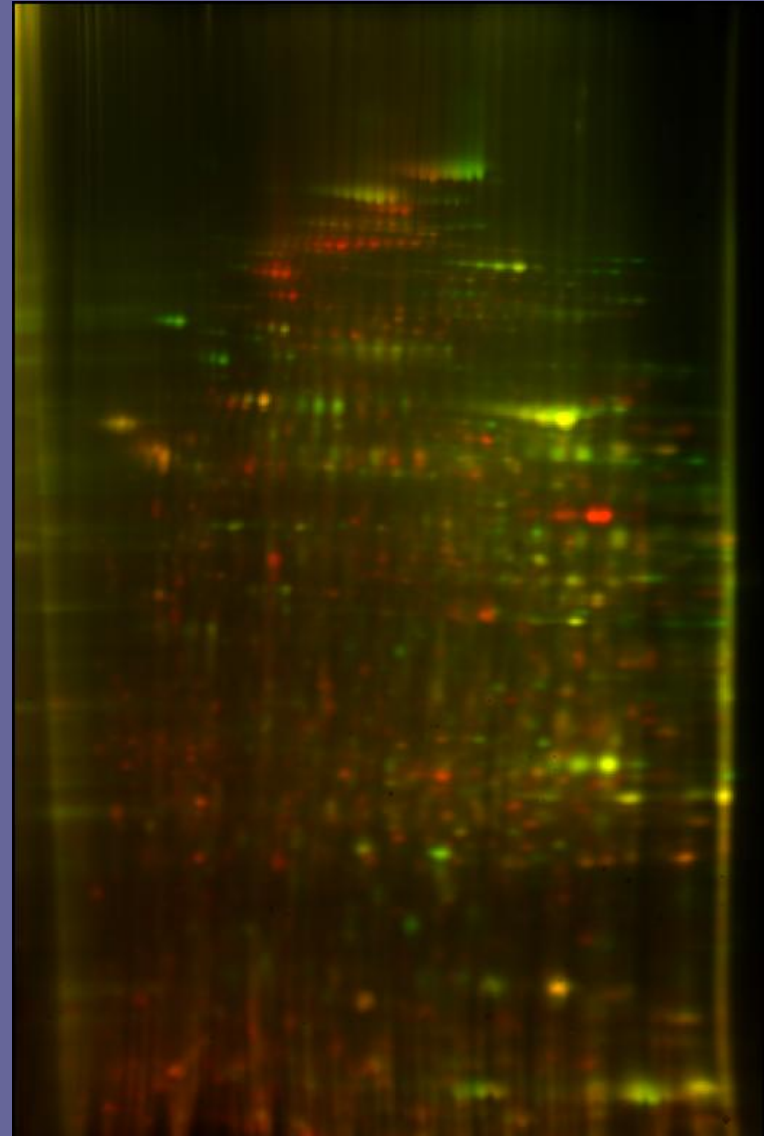
Increased levels of ALT



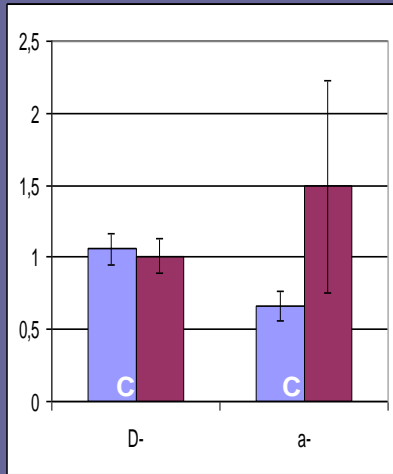


Mitochondria

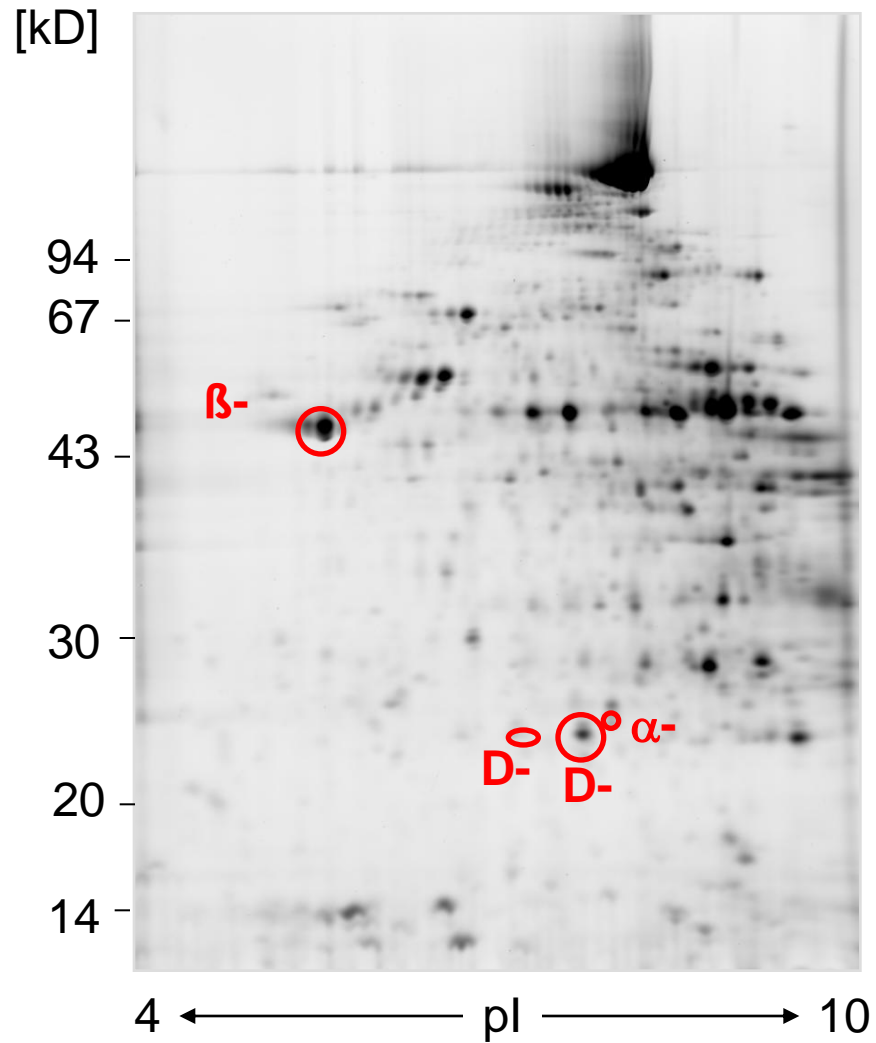
green : control
red: LPS



ATP-Synthase



ATP-synthesis



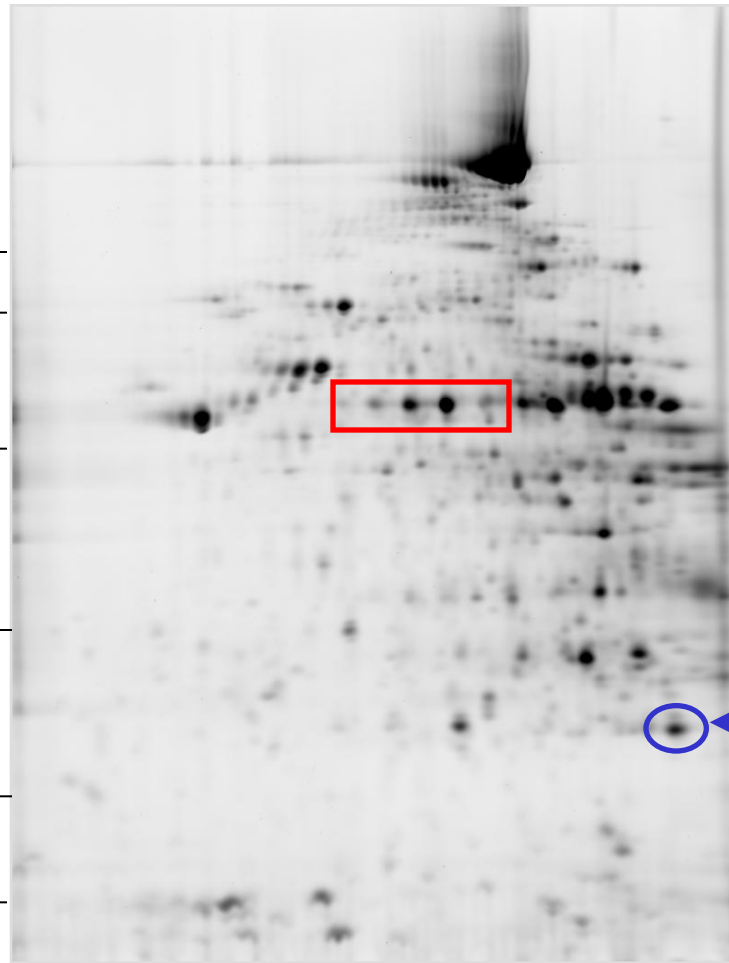
more fragments of carbamoylphosphate synthase

Mitochondrial superoxide dismutase (SOD [Mn])

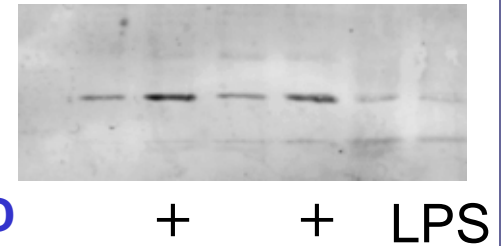
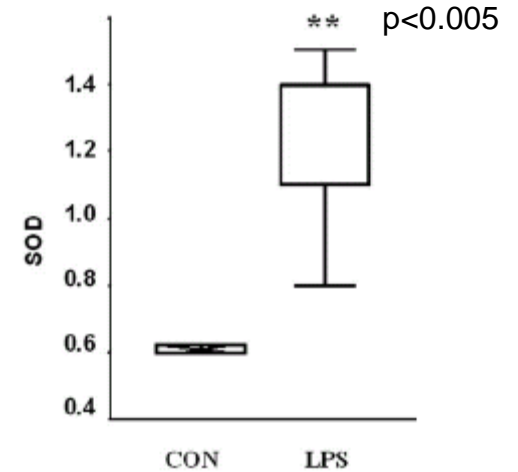
aldehyde
dehydrogenase
polymorphism?

[kD]

94
67
43
30
20
14



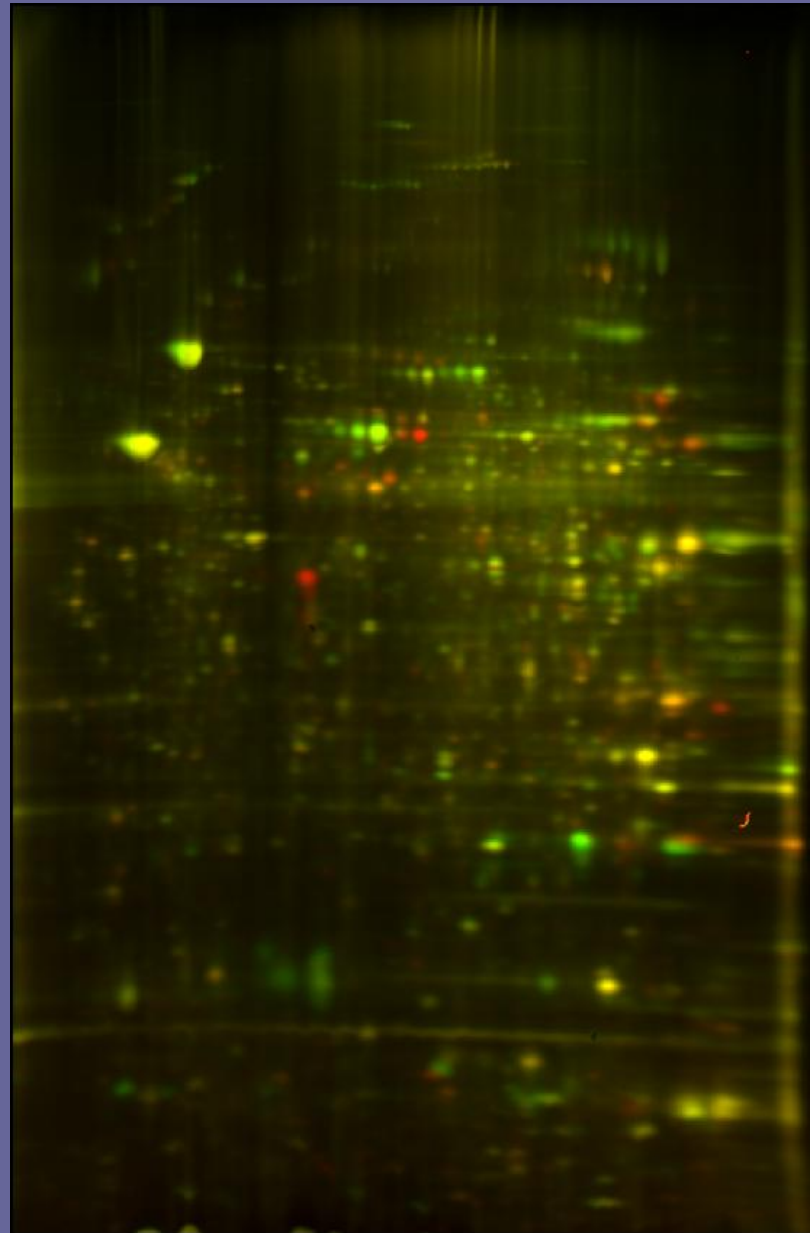
4 ← pI → 10



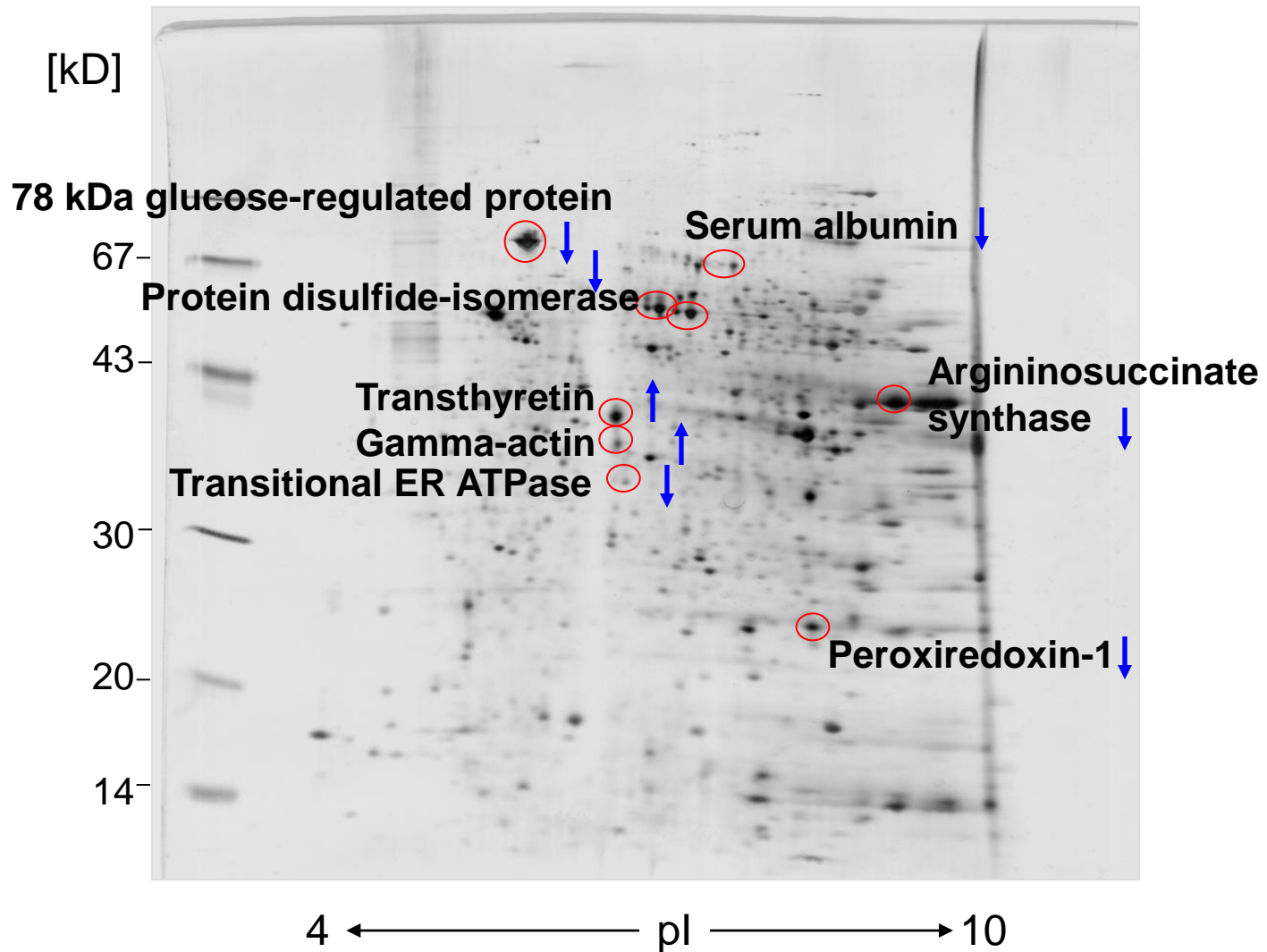
protection
against toxic
radicals

Endoplasmic reticulum

green : control
red : LPS



Differentially regulated ER protein spots



e.g. transport, folding, oxidation, acute phase

Testing of **function**:
function of mitochondria improved,
function of ER decreased.

ROS production

histological changes

**More (pronounced) changes
in endoplasmic reticulum (ER)**

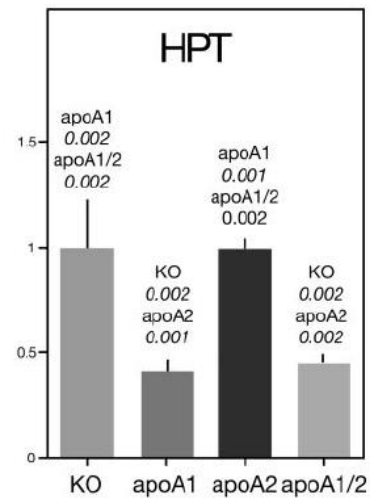
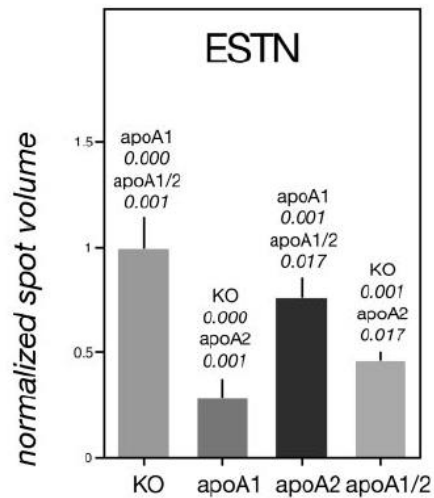
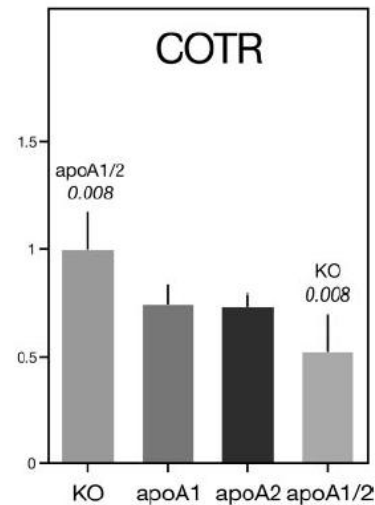
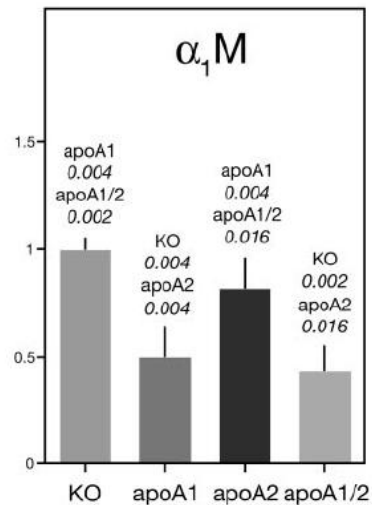
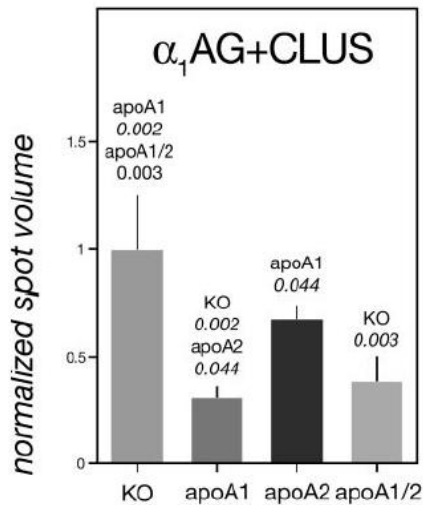
**ER is much more susceptible
to endotoxic shock
than mitochondria**

2. Transgenic animals (mouse)

Apolipoproteins

knock-out: murine Apo A-I and/or II

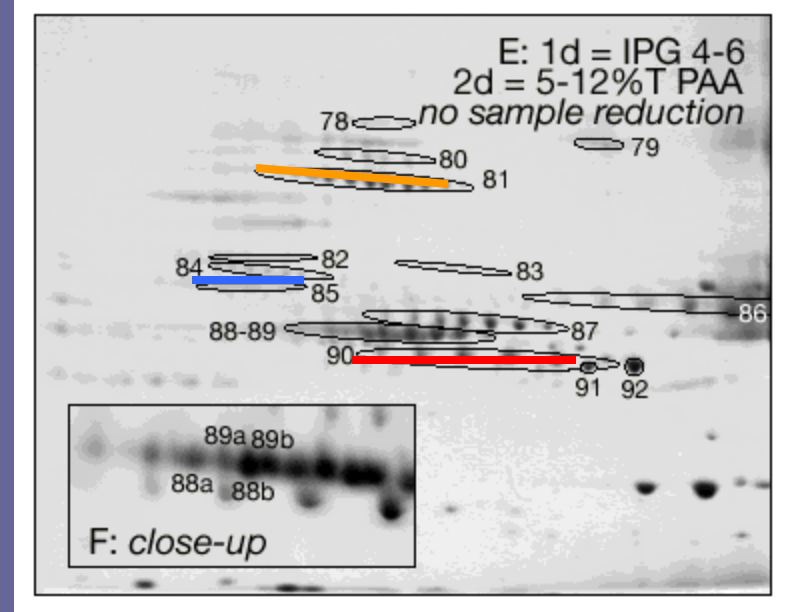
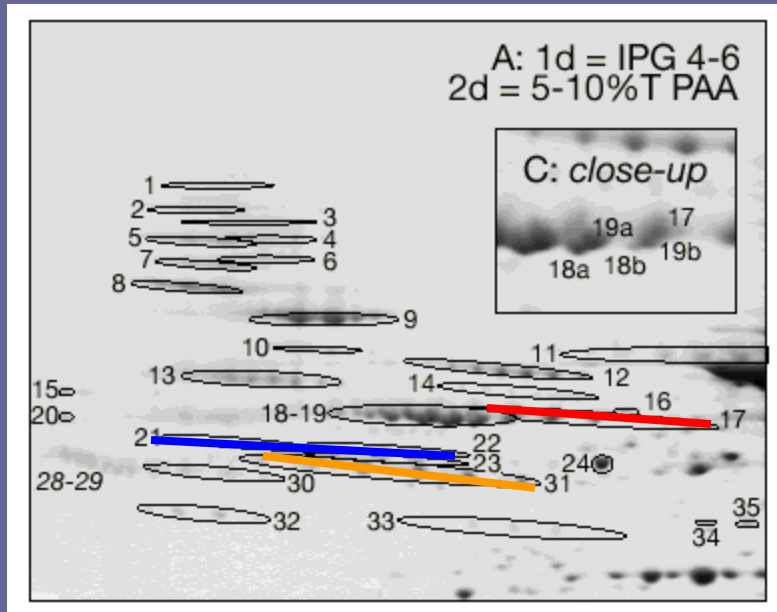
knock-in: human Apo A-I and/or II



mouse strain

Additional changes of:
 orosomucoid + clusterin,
 α_1 -macroglobulin, contrapsin,
 carboxylesterase, haptoglobin.

Improved resolution of relevant proteins by varying running conditions



reducing

conditions

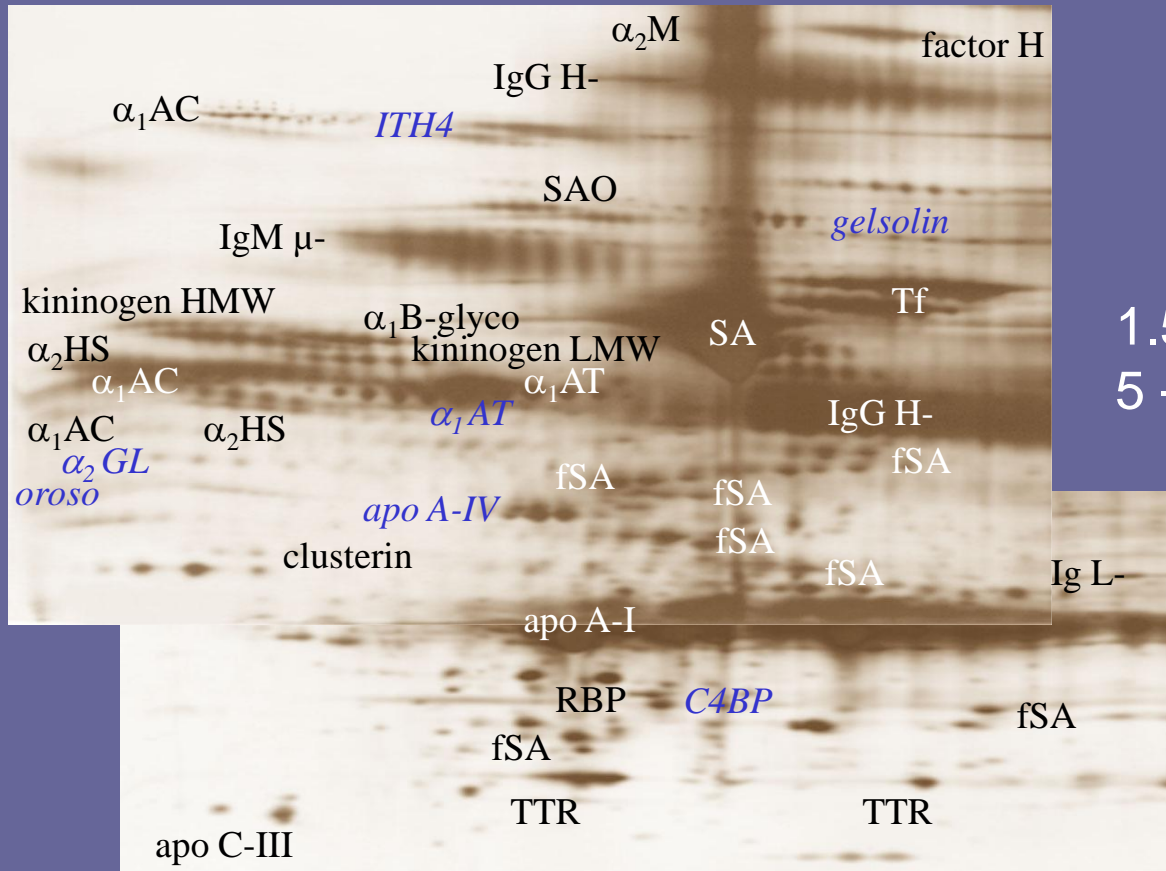
nonreducing

contrapsin

haptoglobin

α_2 -HS-glycoprotein

3. Physiological changes (cow pregnancy)

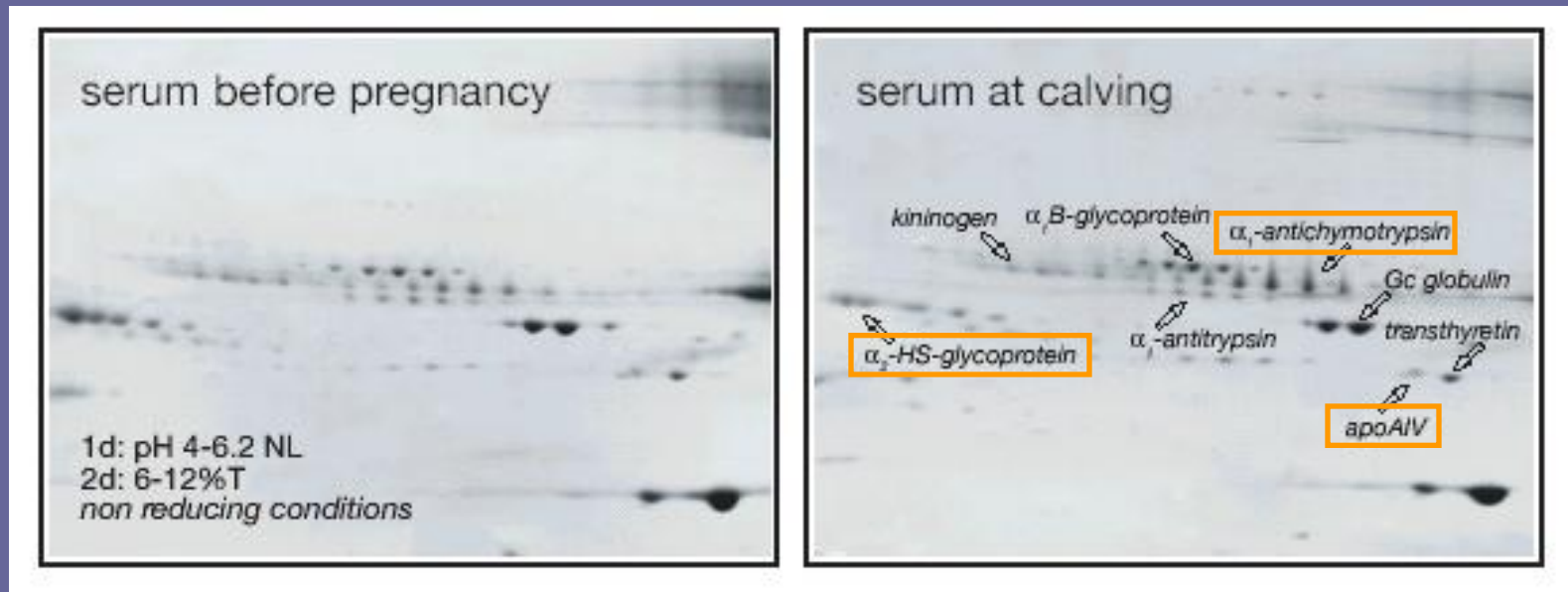


1.5 µl serum
5 - 8 % T

4.5 µl serum, 7.5 - 17.5 % T

Cow serum protein map

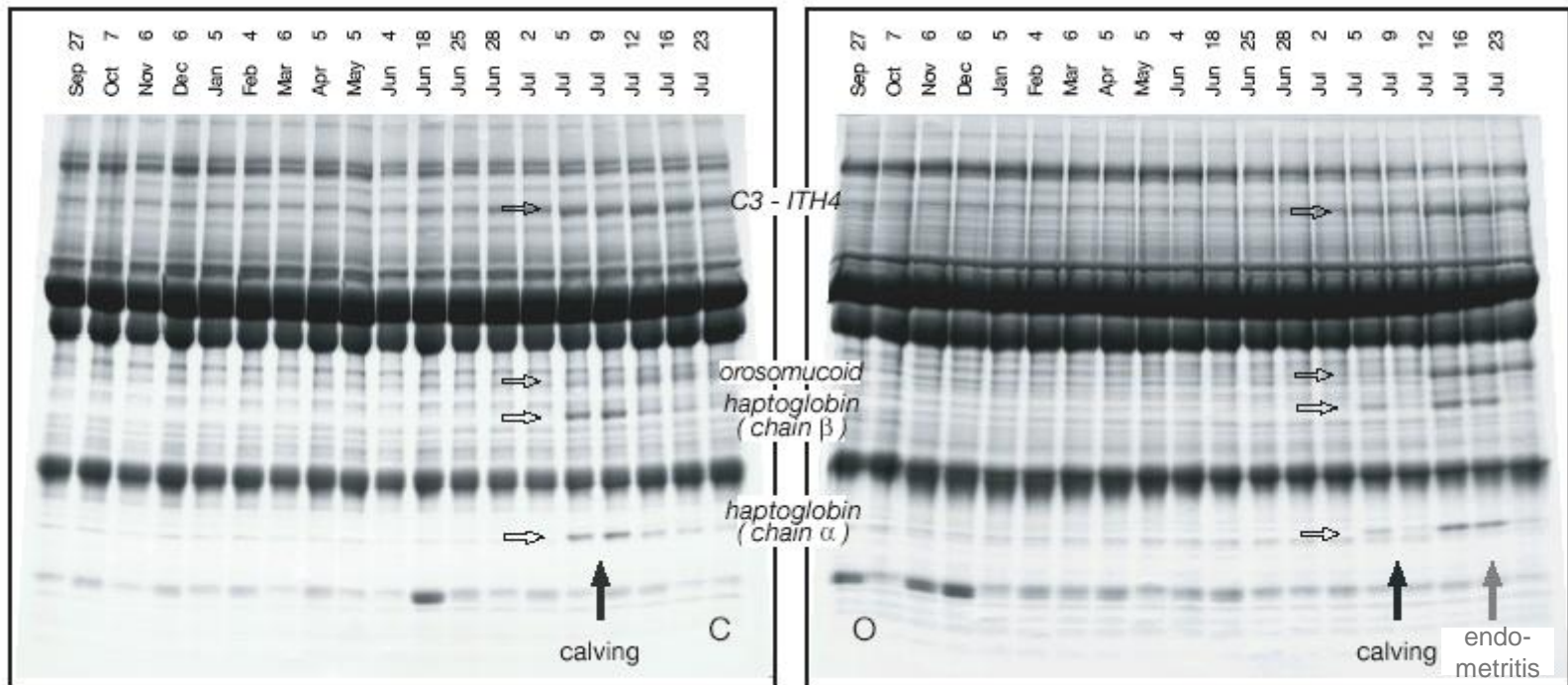
Cow pregnancy



acidic zoom gels, non-reducing conditions

SDS-PAGE

time course

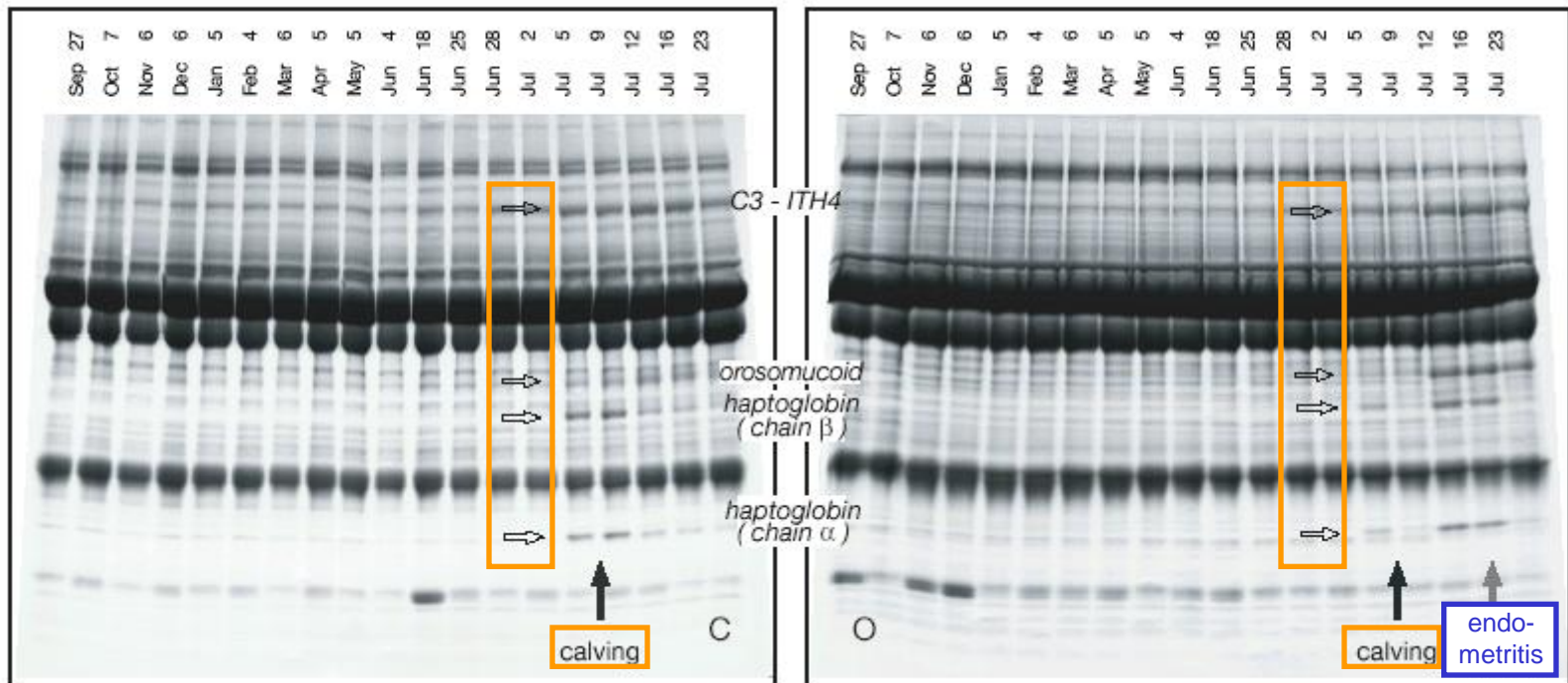


healthy animal

endometritis

SDS-PAGE

time course

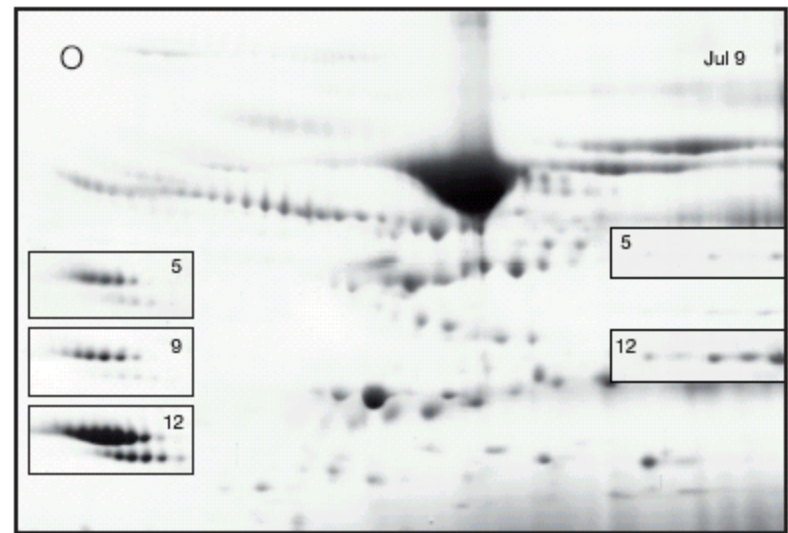
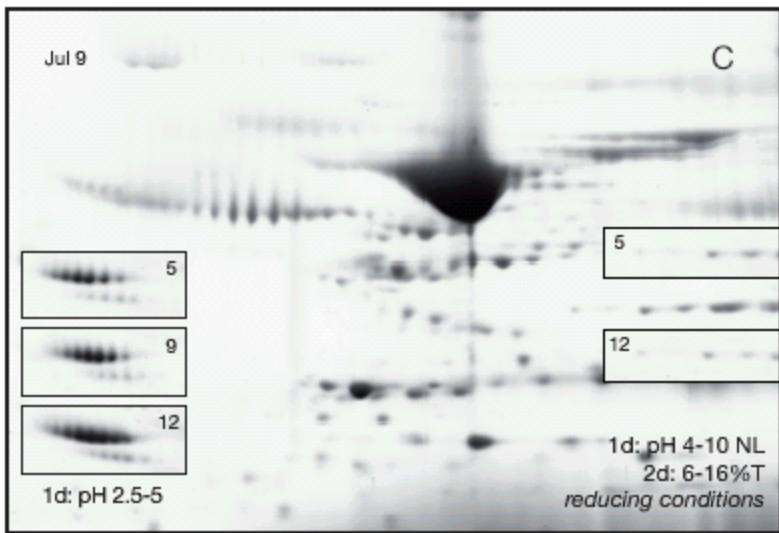
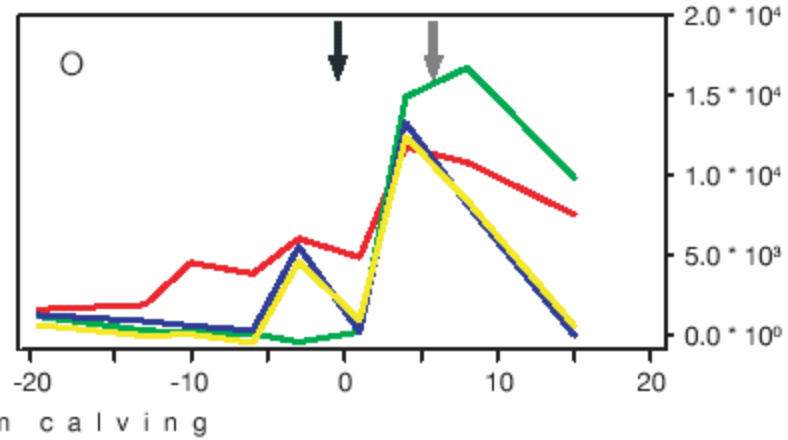
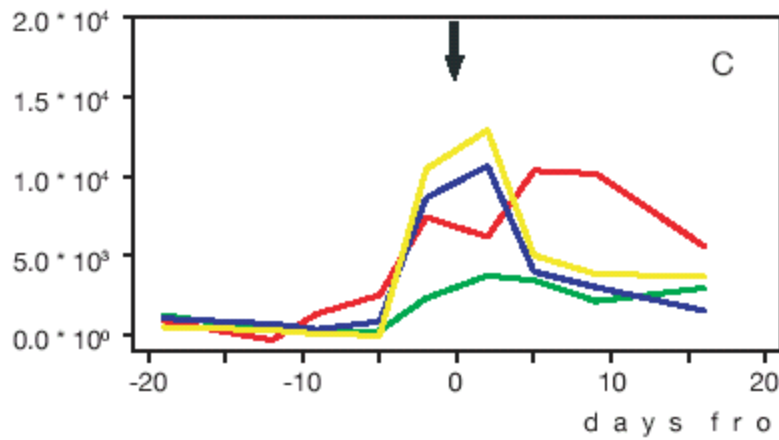
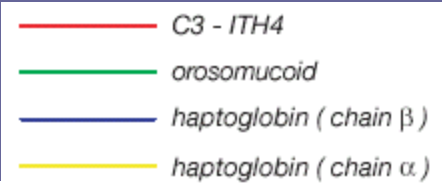


healthy animal

endometritis

healthy animal

endometritis

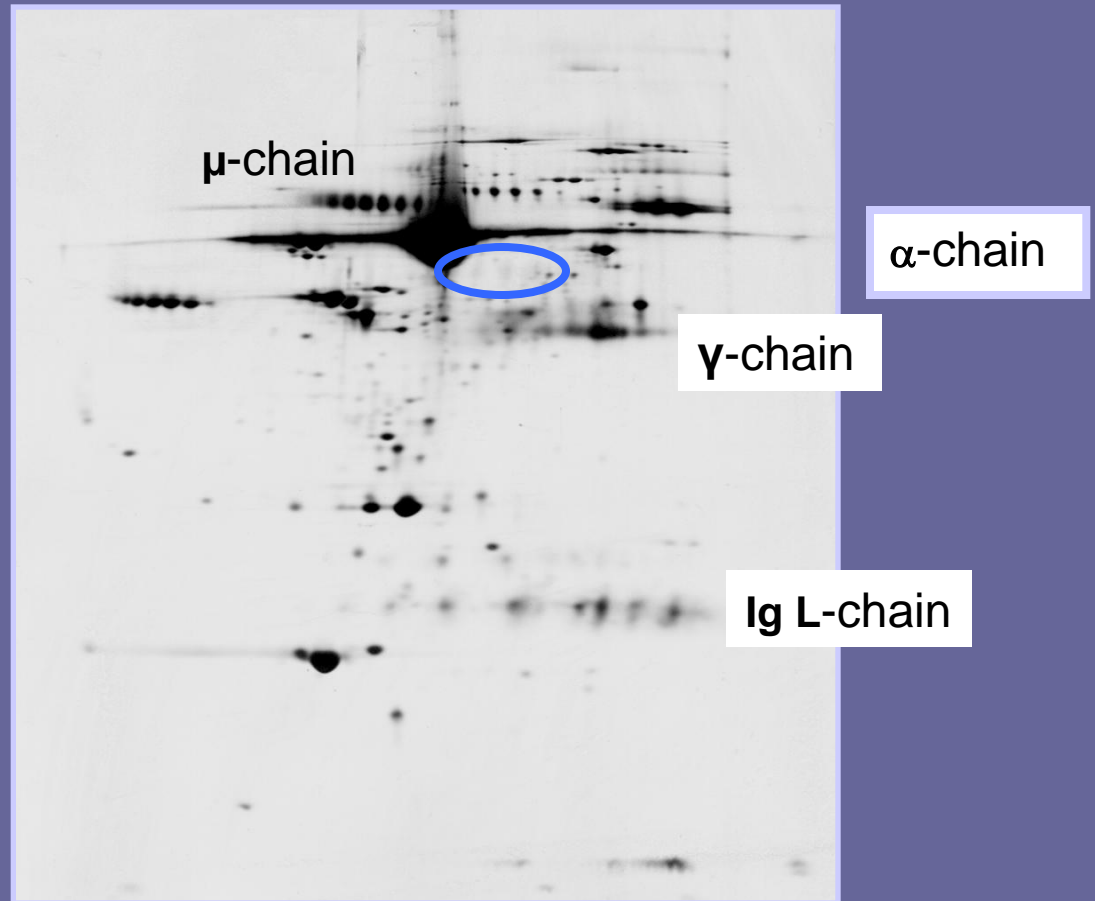


orosomuroid

haptoglobin β -

4. Disorders (gammopathy, dog)

Normal immunoglobulin pattern

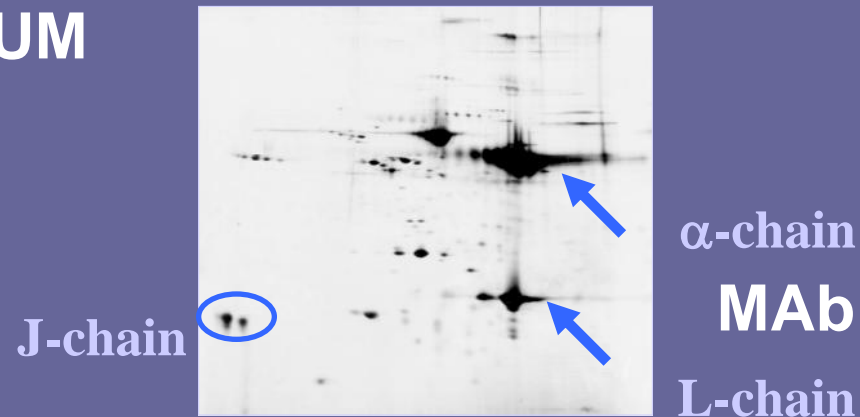


dog serum
(healthy dog)

gammopathies: polyclonal, monoclonal

Multiple myeloma with monoclonal IgA

SERUM

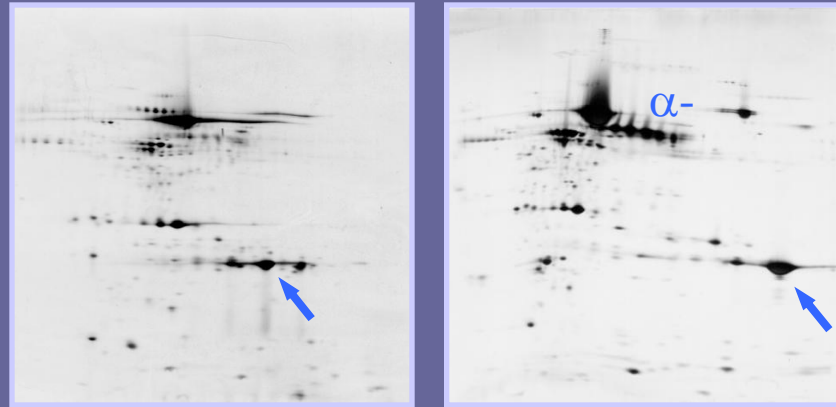


in late stage of the disease / severe cases
also detectable in urine (kidney damage)

Bence Jones Proteins (free Ig L-chains)

URINE

reducing 2-DE



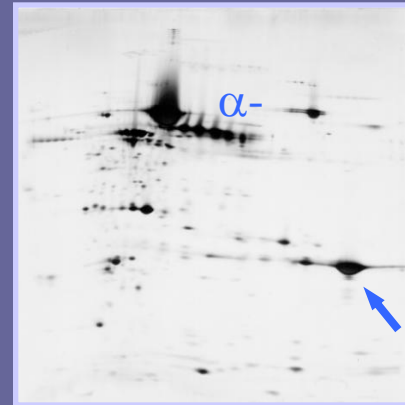
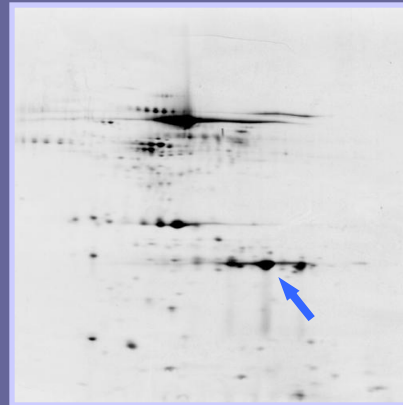
Dog 1:
chronic lymphatic
leukemia;
mIgA and mIgG
in serum

Dog 2:
plasmacytoma;
dimeric BJP +
mIgA in serum

Bence Jones Proteins (free Ig L-chains)

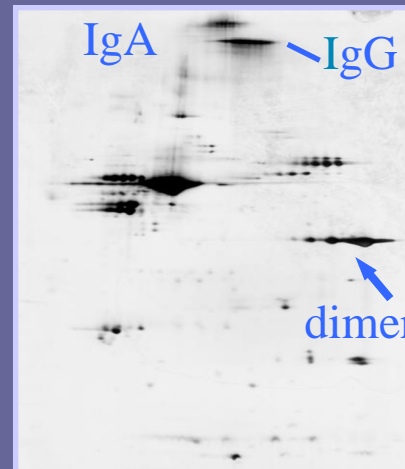
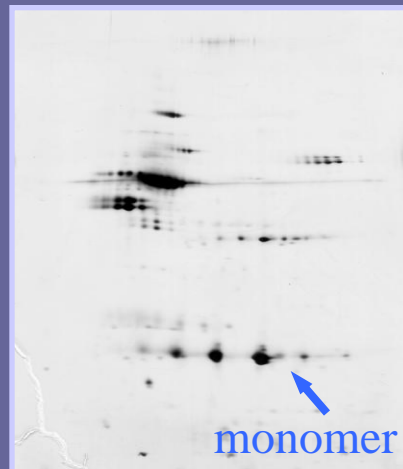
URINE

reducing 2-DE



Dog 1:
chronic lymphatic
leukemia;
mIgA and mIgG
in serum

Dog 2:
plasmacytoma;
dimeric BJP +
mIgA in serum

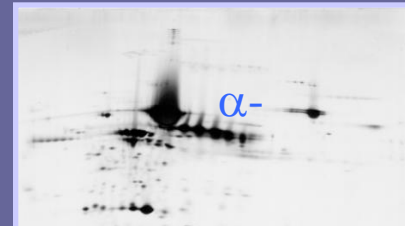
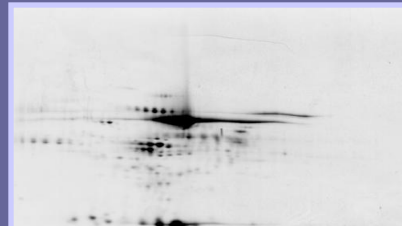


non-reducing 2-DE

Bence Jones Proteins (free Ig L-chains)

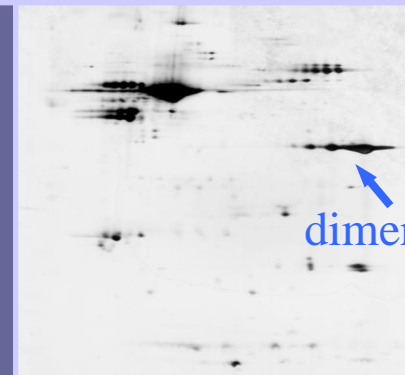
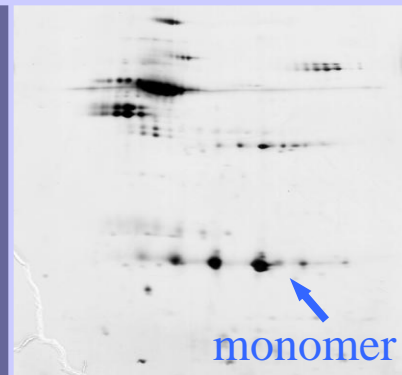
URINE

reducing 2-DE



**non-reducing 2-DE:
for the detection of single subunits,
incomplete molecules**

in serum



in serum

non-reducing 2-DE

UREA

DTT

native

*unfolding
without reduction*

*unfolding
with reduction*

3



2



1



0



Different combinations

native (non-denaturing) IEF / native PAGE

native IEF (non-denaturing) / SDS-PAGE

native IEF (non-denaturing) / red. SDS-PAGE

denaturing IEF / SDS-PAGE

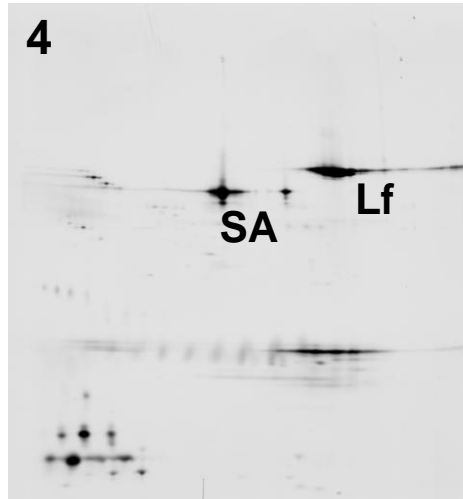
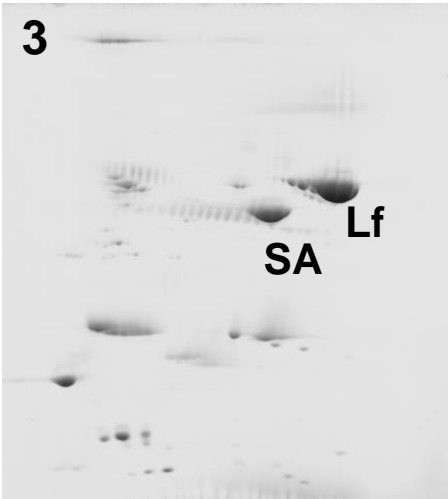
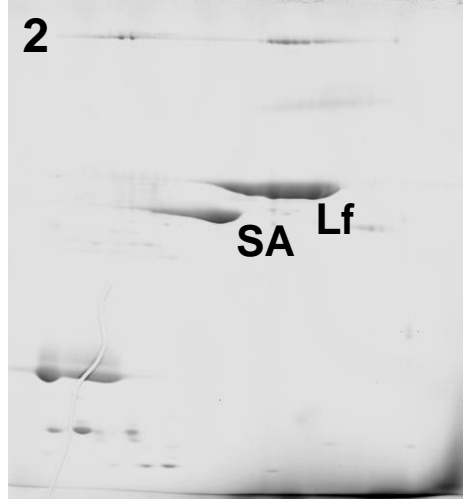
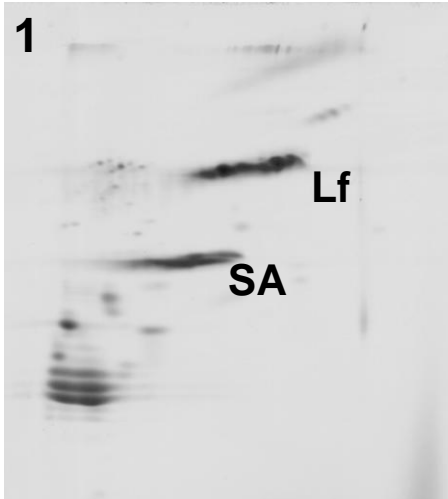
red./denat.IEF / red. SDS-PAGE

2-DE

....

non-denaturing IPG
native PAGE

non-denaturing IPG
SDS-PAGE



Rabbit milk

IPG (urea)
SDS-PAGE

IPG (urea, DTT)
SDS-PAGE (DTT)

5. Homologous proteins (fibrinogen)

Structure of fibrinogen

340 kD plasma glycoprotein

Structure of fibrinogen

340 kD plasma glycoprotein

3 pairs of polypeptide chains:

α -, β -, γ -

linked by disulfide bonds
and stabilized by Ca^{2+}

Approaches for identification:

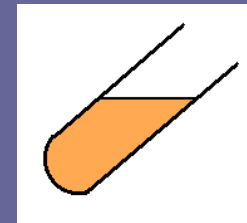
a) comparison serum/plasma

b) immunoblotting

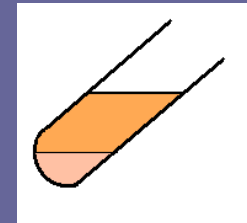
Approaches for identification:

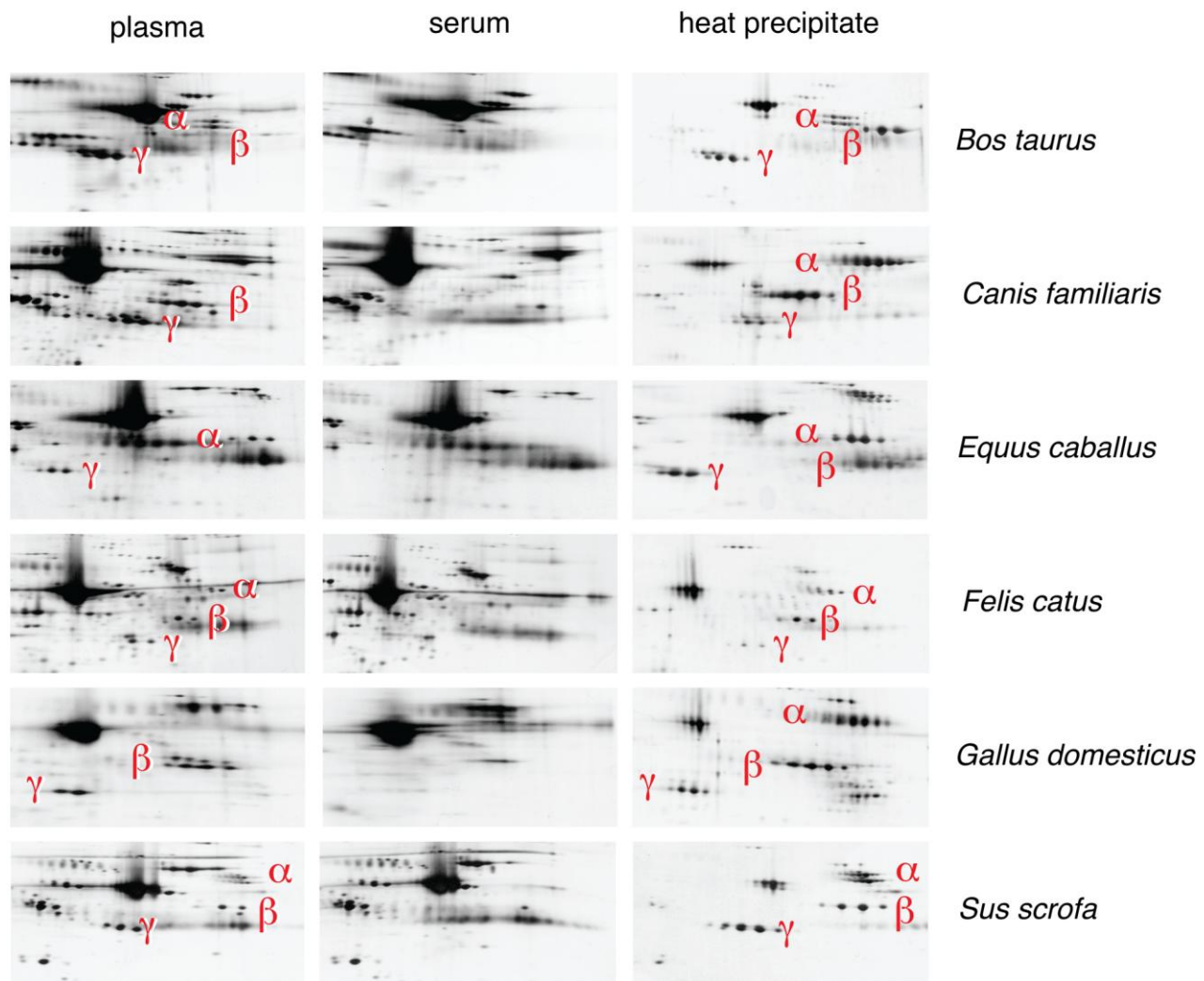
c) heat precipitation

dissolve and analyse
precipitate in 2-DE

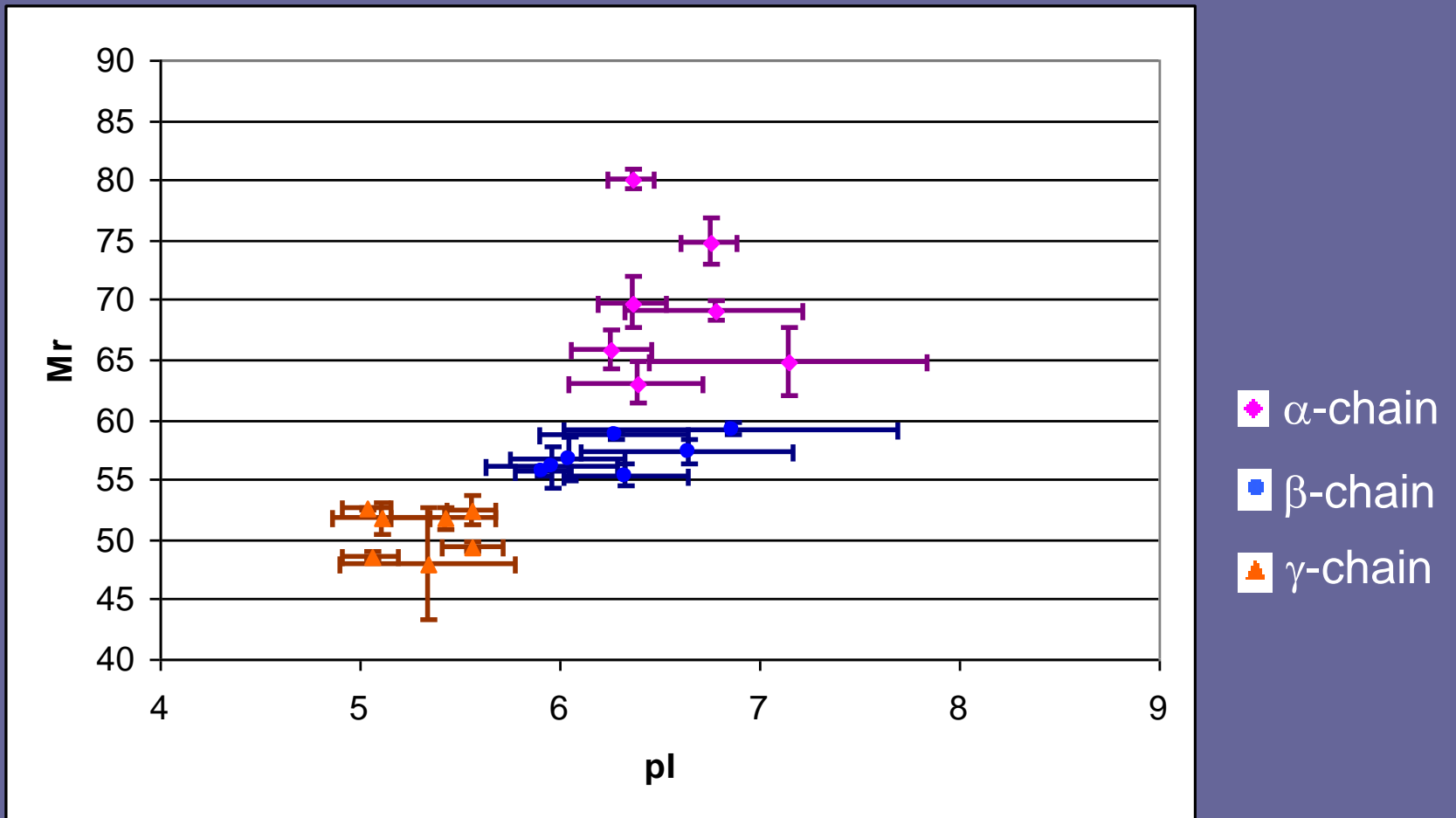


56°C
3-10 min



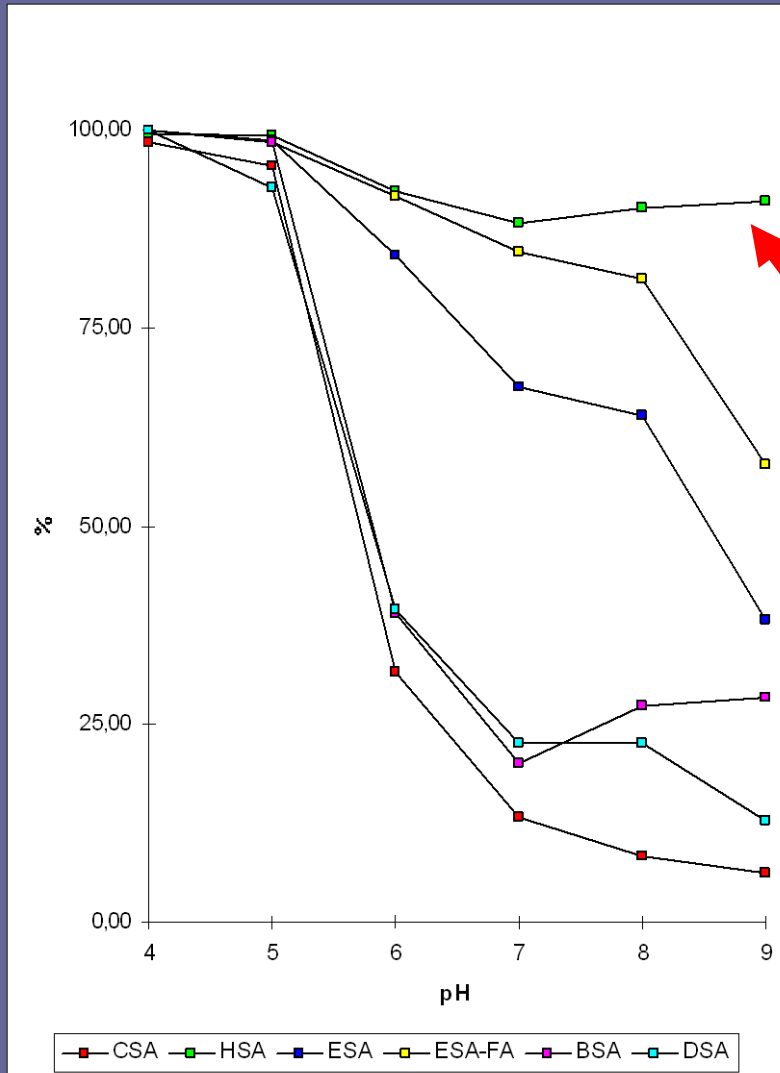


Physicochemical data of fibrinogen chains of different species



6. Depletion of high-abundance proteins (albumin)

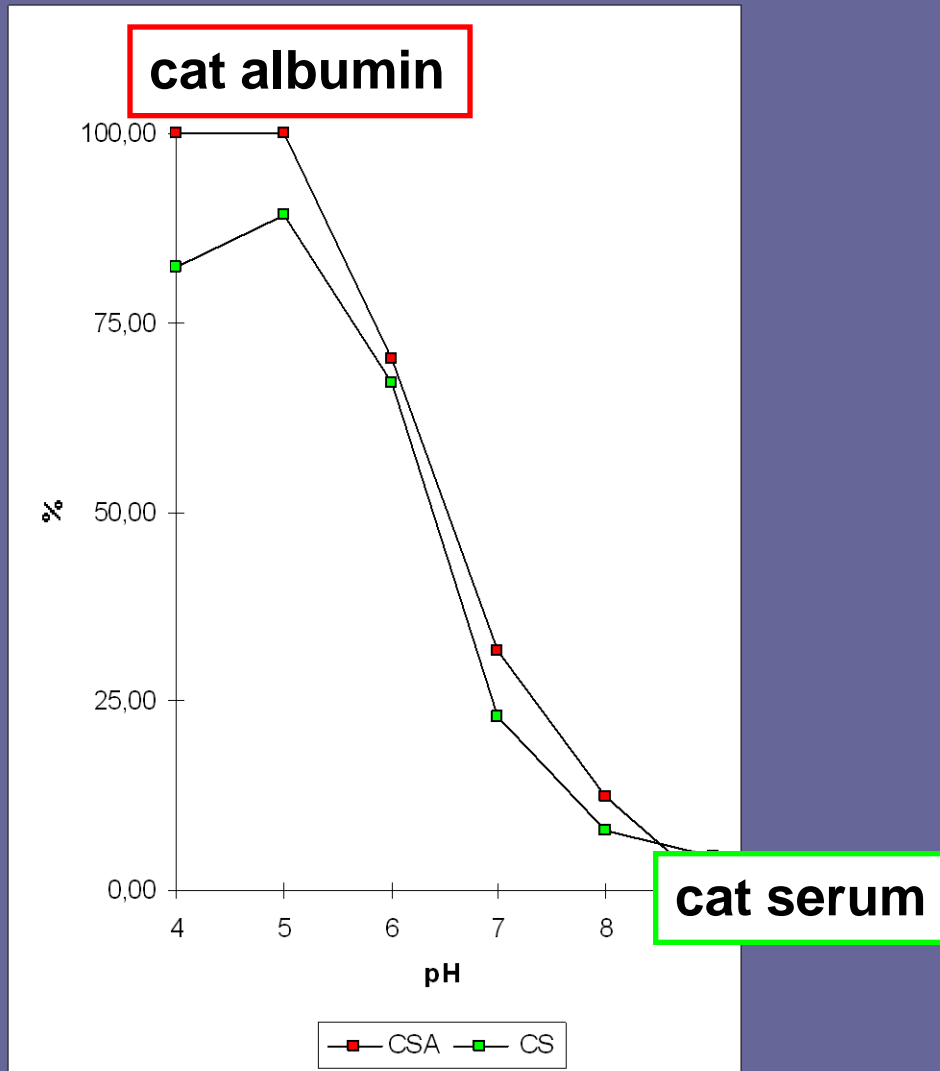
Albumin binding to Cibacron Blue F3G A



albumins of different species bound on a "blue column" as a function of pH

better binding and higher capacity for HSA

Albumin binding to Cibacron Blue F3G A



binding of all serum proteins increases at lower pH

University of Veterinary Medicine, Vienna

LBI for Exp. & Clin. Traumatology, Vienna

Medical University, Vienna

University of Graz

Università degli Studi, Milano

Kennedy Institute, London

and all the others...

Thank you!



...and you!

Thank you!