System-Level Analysis of Proteolytic Events in Increased Vascular Permeability and Complement Activation in Skin Inflammation

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Ulrich auf dem Keller

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+ "Identifying and quantifying proteolytic events and the natural N terminome by terminal amine isotopic labeling of substrates", Nat Protoc., 2011 , Sep 22;6(10)

Journal Club 1, 19.03.2013

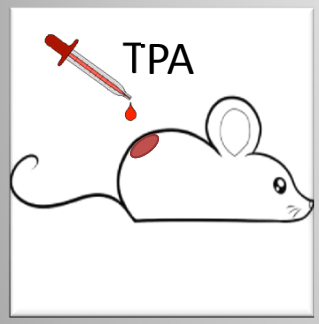
Yakushev Sergey

Outline

1. Background
2. Motivation for the study, aims
3. Study design, Models, Methods
4. Results
5. Discussion: Significance and relevance of the results
6. Possible implication in projects of Prof. Dr. Aguzzi

Background

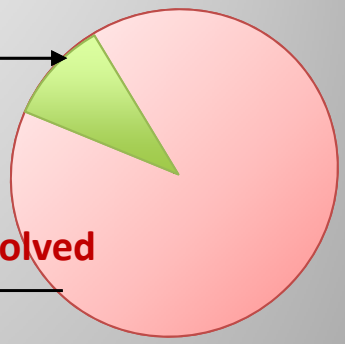
Inflammation



Proteases and their inhibitors (664 genes in mice):

Kininogen → bradykinin
proChemokines → Chemokines

well studied



potentially innvolved

Cell migration
Extracellular matrix remodeling
Removal of the debris

Hard to study proteases: functional information

- Protease aboundance and localization
- Protease activity
- Many potential substrates
- Interconnected activity

To study the role of even single protease:

Functional information of a large-scale

Motivation for the study and aims

1. Development of the high-content screening tool:
functional information on proteases on a large scale

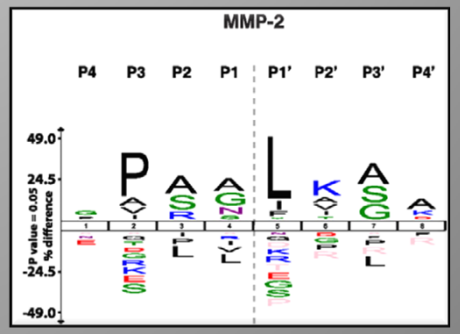
Ultimate goal - to generate hypotheses as a basis for more detailed studies that aim to confirm in vivo mechanisms

2. Screen global changes in proteolytic modification of the inflammatory proteome in vivo

complex protease function in health and disease

3. Dissect the impact of a single protease MMP2 on proteolytic signature of skin inflammation

MMP2:
- immunomodulatory, associated with tissue repair, angiogenesis
- multiple potential substrates

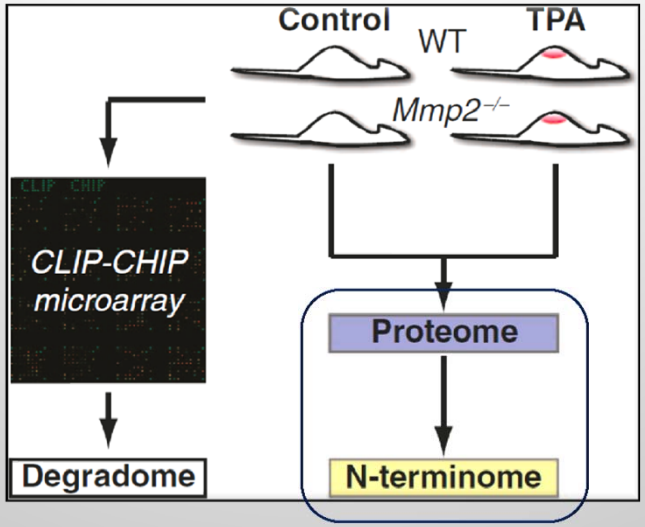


A. Prudova, 2010, Mol&Cel Proteomics

Study design, Models, Methods

CLIP-CHIP: Transcriptome
All proteases, non-proteolytic homologs, and inhibitors gene transcripts
aka **DEGRADOME**

	Aspartic	Cysteine	Metallo	Serine	Threonine	Inhibitor
Human	21 (0)	151 (16)	187 (40)	178 (26)	28 (12)	156 (17)
Murine	27 (4)	162 (14)	205 (43)	226 (28)	26 (12)	197 (17)



In vivo model
TPA induced skin inflammation
48h

WT and MMP2$^{-/-}$

Frustrating note on animal model (♀):
2 WT TPA vs 2 WT control
2 KO TPA vs 2 KO control
Only 2 replicates!

«Extracellular fraction» isolation: 100 mM HEPES pH8.0

Taging iTRAQ (isobaric tags for relative and absolute quantification)

Proteome: Shotgun proteomics (HPLC + nanoLC-MS/MS)
N-terminome: iTRAQ-TAILS (iTRAQ-Terminal Amine Isotopic Labeling of Substrates)

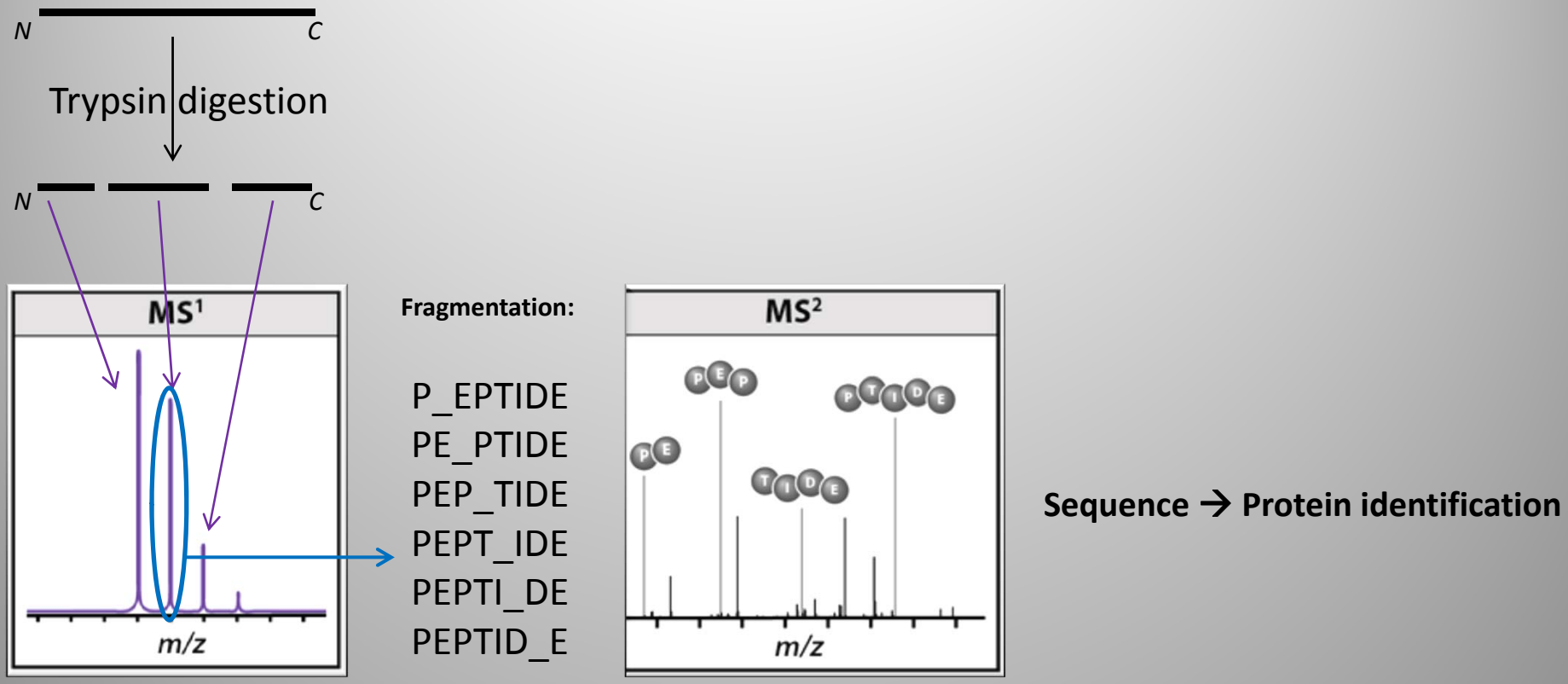
Data analysis:

1. Search (mouse International Protein Index datatabase)
2. Evaluation (Trans-Proteomic Pipeline)
3. Quantification
4. Statistical analyzis

Validation of the selected events

Study design, Models, Methods

MS/MS



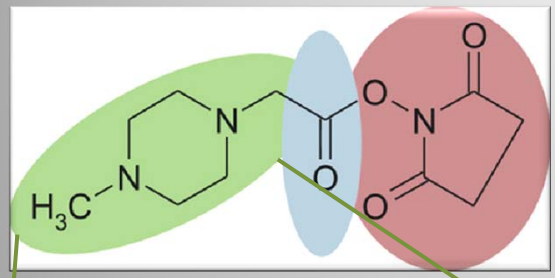
Sequence → Protein identification

Mixture of proteins: Shotgun proteomics

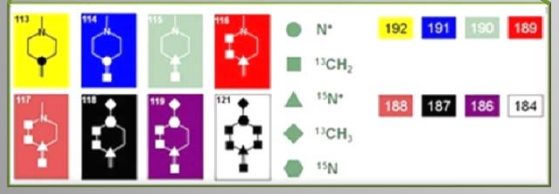
- tissue lysates are too complex, identification of the most aboundant proteins
- «yes/no» information

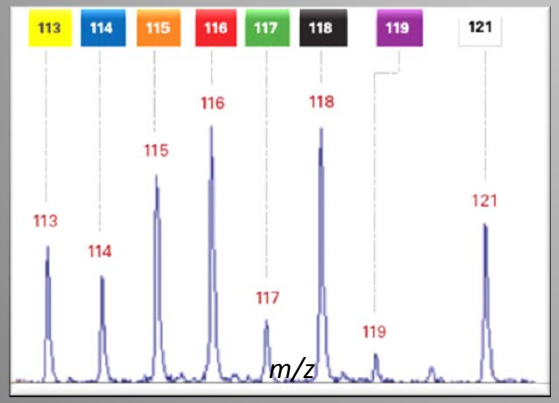
Study design, Models, Methods

iTRAQ (isobaric Tags for Relative and Absolute Quantification)

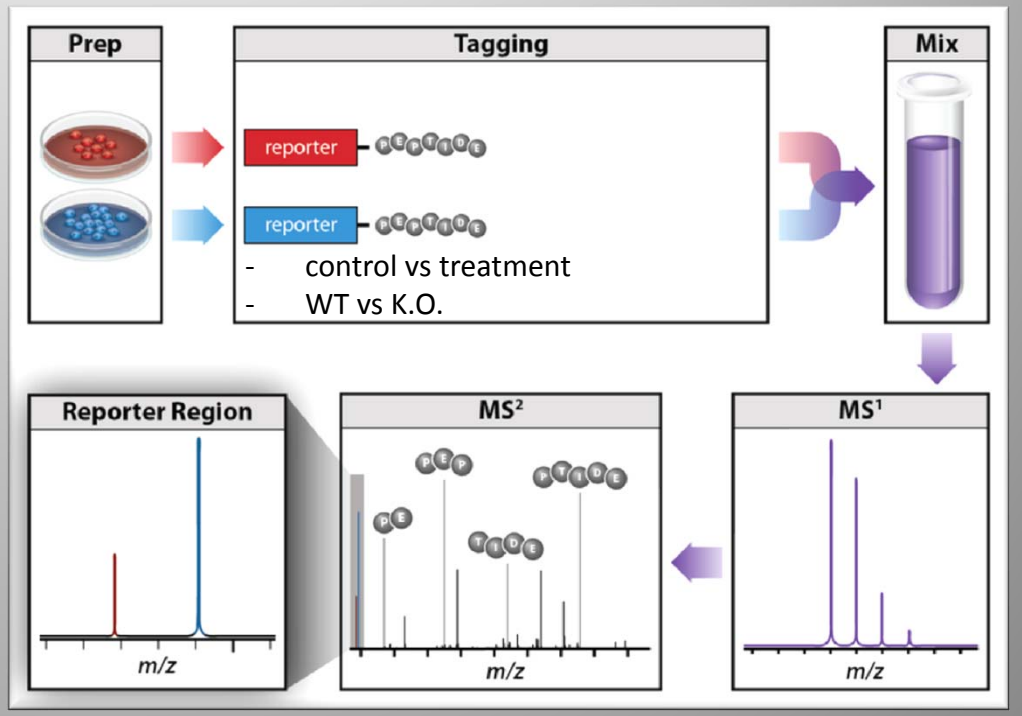


Quantification group





Reacts with
N-terminal amino groups
and
amino group of lysine

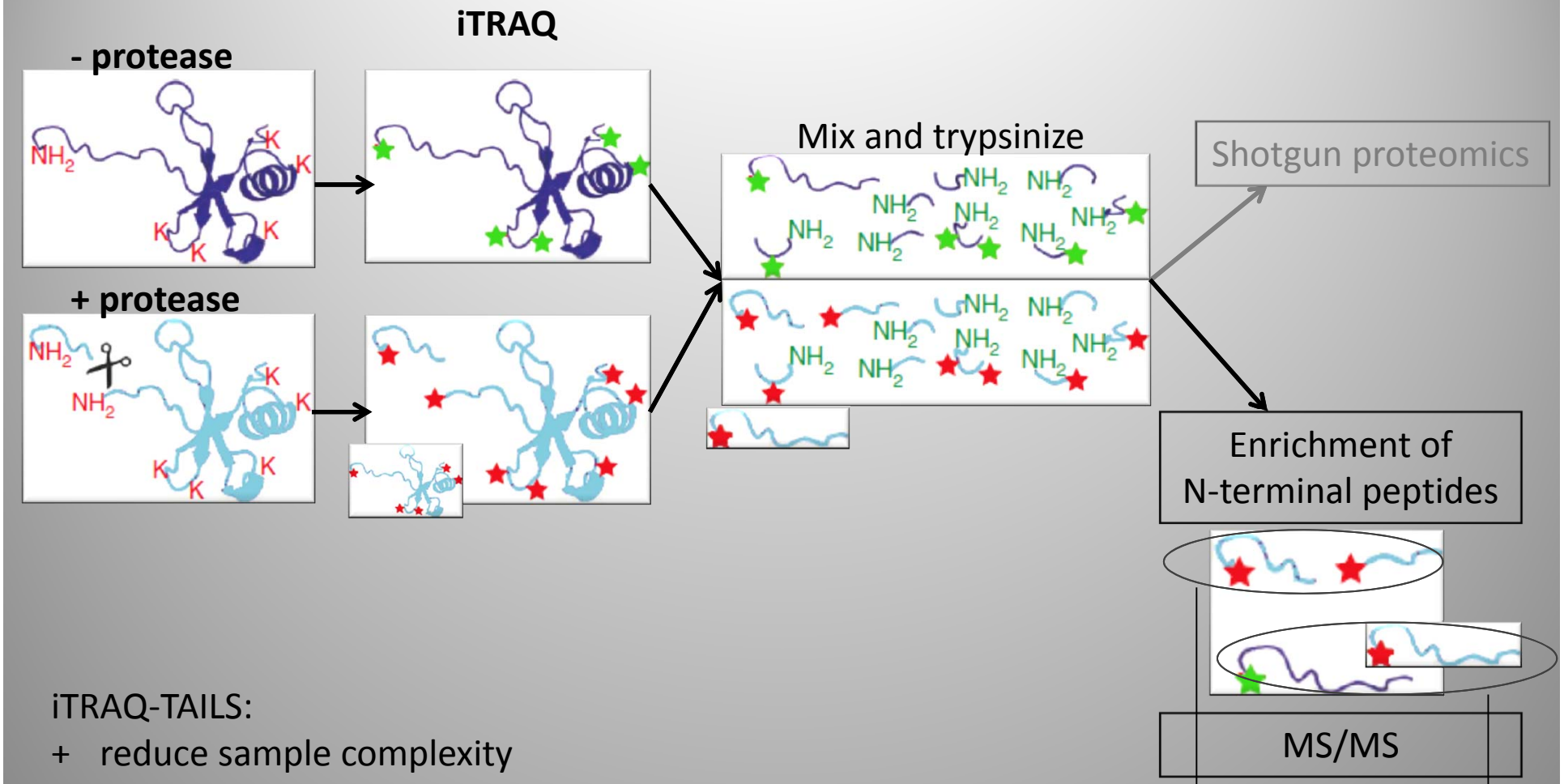


Proteomics: yes/no

iTRAQ: how much

Study design, Models, Methods

iTRAQ-TAILS (Terminal Amine Isotopic Labeling of Substrates)



iTRAQ-TAILS:

+ reduce sample complexity
+ identify cleavage spectrum, cleavage sequence
+ quantify proteolysis of specific target
+ identify and often quantify N-acetylation

100-300 mkg of tissue

Study design, Models, Methods

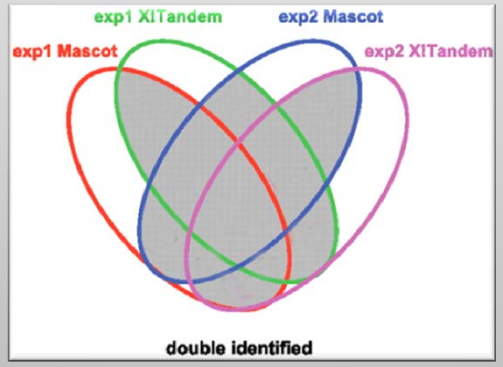
Data analysis

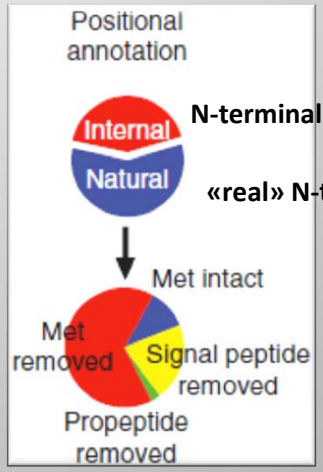
Search (at least 2 databases) **Mascot** and **X! Tandem**

List of all possible proteins

Evaluation (Trans-Proteomic Pipeline) + Quantification

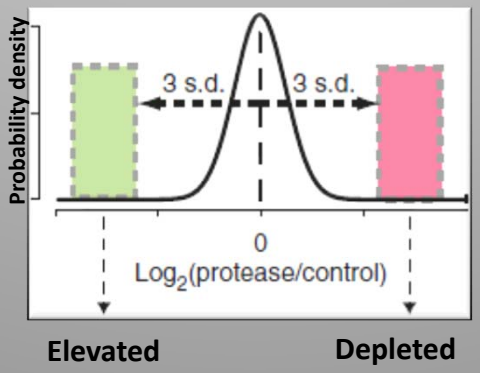
High-confidence identification



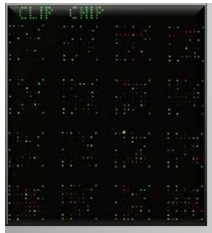


Statistical analyzis

$\log_2([\text{treatment}]/[\text{control}])$



A Statistics-based Platform for Quantitative N-terminome Analysis and Identification of Protease Cleavage Products, 2010, auf dem Keller, Mol&Cell Proteomics

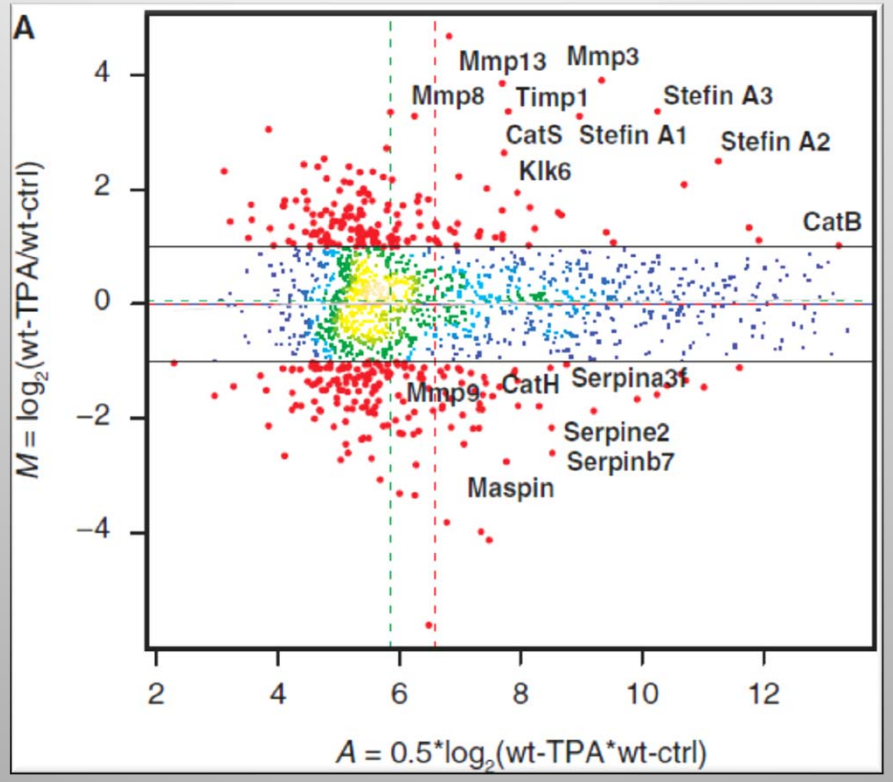


CLIP-CHIP

Results

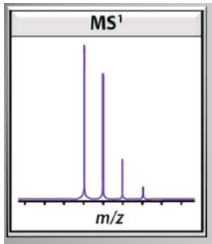
Degradome

$WT_{control}$ vs $WT_{inflammation}$ (208 altered significantly)



↑	↓	≡
Proteases: MMP (3, 8, 13) Cathepsins kallikrein	Proteases: MMP9	Proteases: MMP2
Inhibitors: TIMP, Stefin	Inhibitors: Serpin	

tight regulation of increased proteolysis



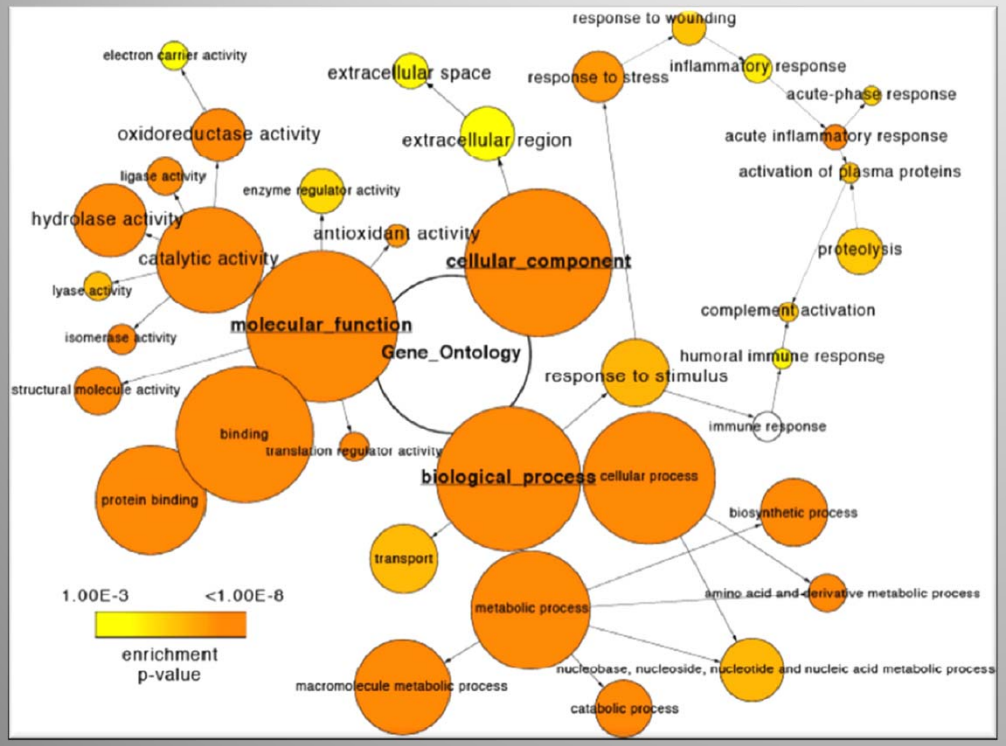
Proteome

Results

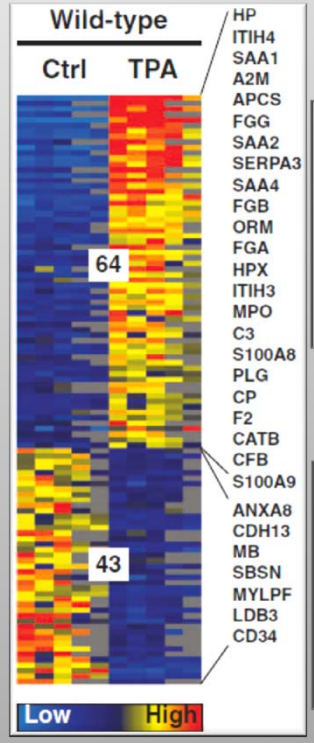
Proteome

$WT_{control}$ vs $WT_{inflammation}$

1147 proteins identified
976 quantified



107 over 2fold changes



Acute phase proteins
Complement
Coagulation
Proinflammatory S100
Myeloperoxidase (neutrophil influx)

Annexin 8
Cadherin 13
Suprabasin
Filaggrin 2
(hyperproliferative response)

Infiltration of acute phase proteins
Hyperprolyferative state

N-terminome

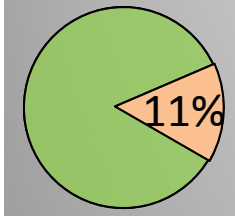
Results

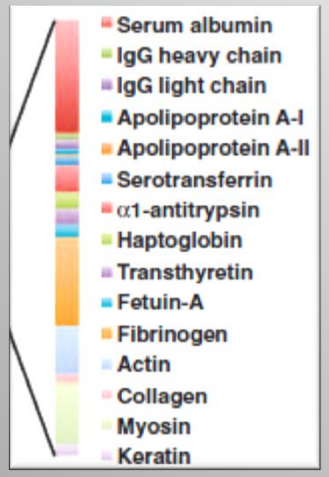
N-terminome (iTRAQ-TAILS)

$WT_{control}$

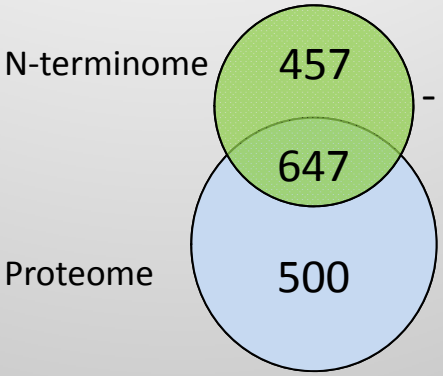
Curse of all proteomics:
high aboundant proteins

N-terminome



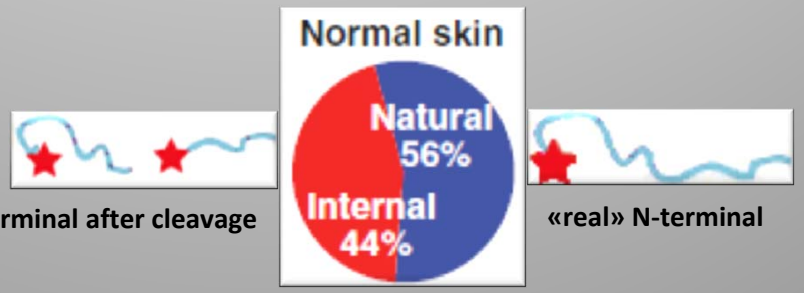


1813 N-termini, 1104 proteins



CTSL
CCL6
CXCL2
CXCL5
CXCL7
IGFBP3
IGFBP4
LTBP4
MSP
MMP3
SDF2

Origin of 1813 N-termini

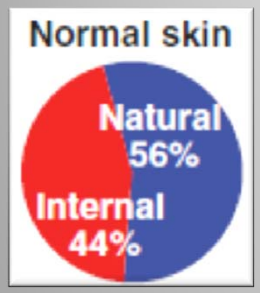


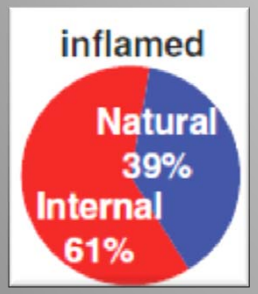
High level of background proteolysis

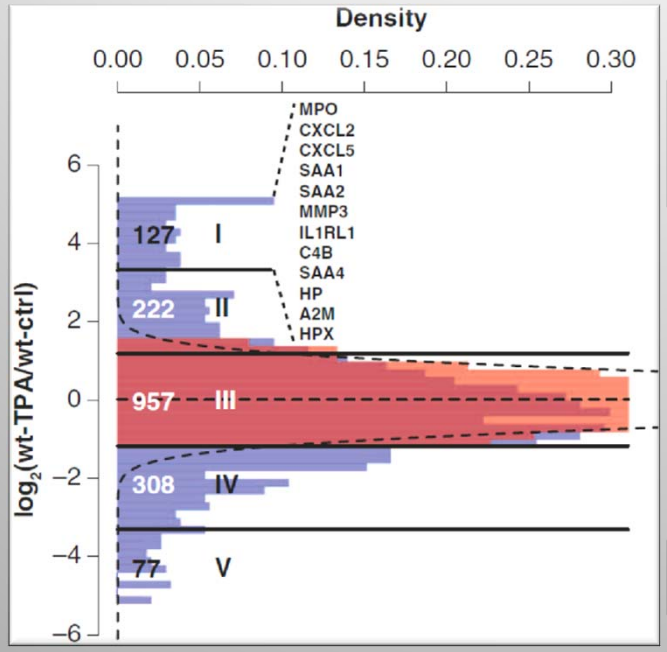
Results

N-terminome (iTRAQ-TAILS)

$WT_{control}$ vs $WT_{inflammation}$







18 proteins appear
(local synthesis or import)

1. **Acute phase proteins from serum**
2. **Produced in epidrmal cells (MMP3)**
3. **Released from resident/infiltrated inflammatory cels**

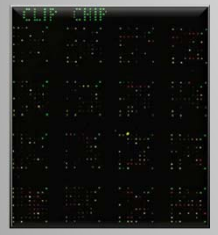
Degradation processes

1. **N-terminal ragging of antithrombin III**
2. **Enzyme activation: plasminogen→plasmin**
3. **Precursor processing: kininogen→bradykinin**
4. **Chemokine processing: CXCL5 – inactivation by signal peptide cl.**
5. **Release of cryptic growth factors from ECM: collagen IV→ arrestin**
6. **S100 protein cleavage (proinflammatory)**
7. **Proteolytic maturation by profilaggrin endoproteinase**

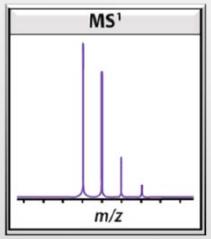
Increased rate of proteolysis

Results

$WT_{control}$ vs $WT_{inflammation}$



CLIP-CHIP



Proteome

N-terminome

Altered mRNA of several proteases and inhibitors:
tight regulation of increased proteolysis

Infiltration of acute phase proteins

Hyperprolyferative state

Increased proteolysis rate
Infiltration of serum/inflammatory cells proteins
Detection of specific cleavage processes

In some cases divergent abundance in mRNA and protein level

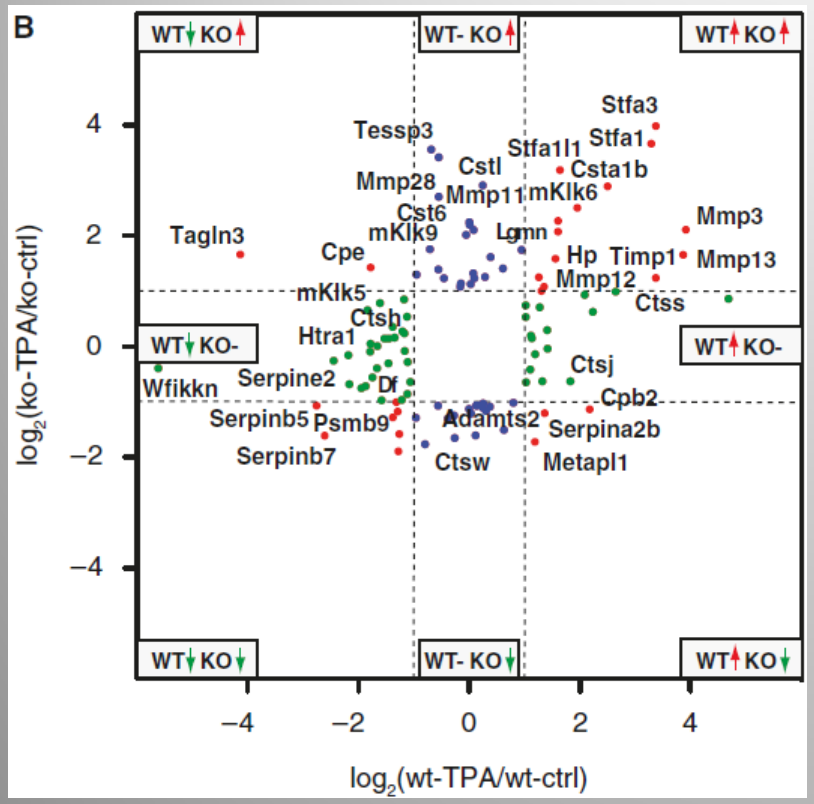
CLIP-CHIP

Results

$WT_{control}$ vs $KO_{control}$

$WT_{inflammation}$ vs $KO_{inflammation}$

KO= $MMP2^{-/-}$



$WT_{control}$ vs $KO_{control}$ (29 altered significantly)

$WT_{inflammation}$ vs $KO_{inflammation}$ (82 altered significantly)

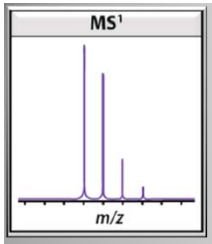
Inflammation:

KO↑WT≡ MMP11, MMP28, CatL, KLK9

WT↑KO≡ CatJ

MMP2 regulation of gene expression

indirect? compensation?

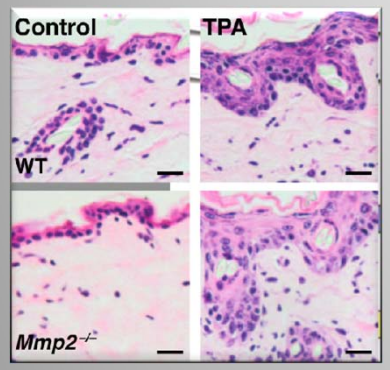


Proteome

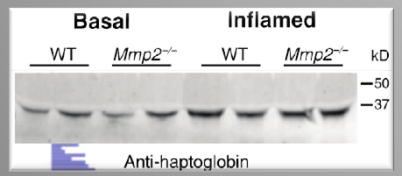
Results

$WT_{control}$ vs $Ko_{control}$

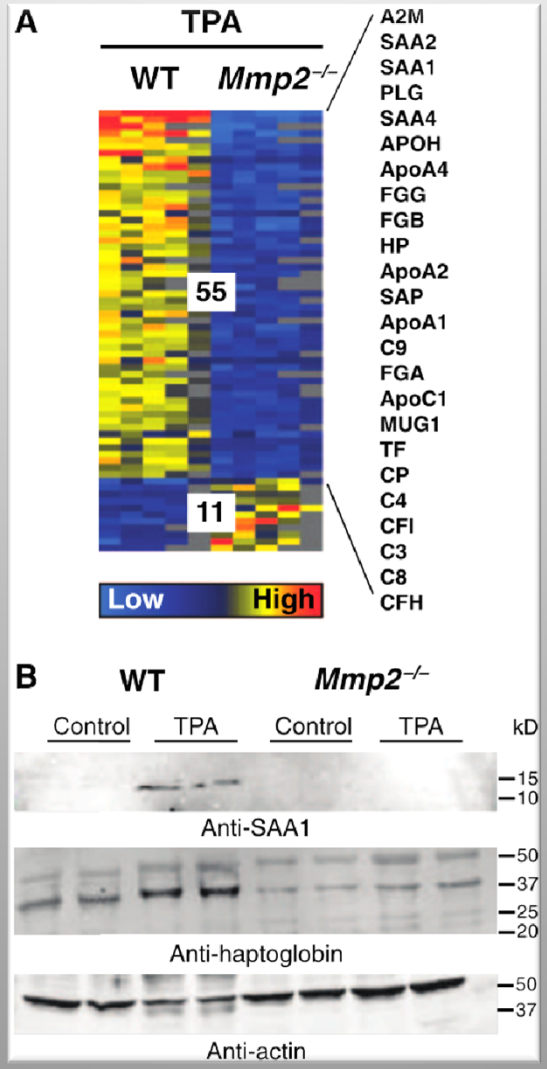
$WT_{inflammation}$ vs $KO_{inflammation}$



Hematoxylin eosin staining.
no differences in the inflammatory cell infiltrate or epidermal hyperproliferation

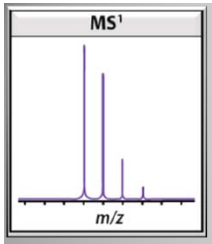


No Haptoglobin changes in liver



Decrease:
Reduction in the extent of exudation of acute-phase proteins, including complement factors and general plasma proteins

Increase:
cell shape, adhesion, junction

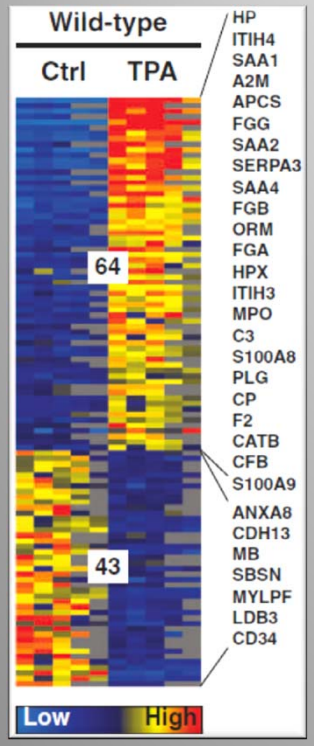


Proteome

Results

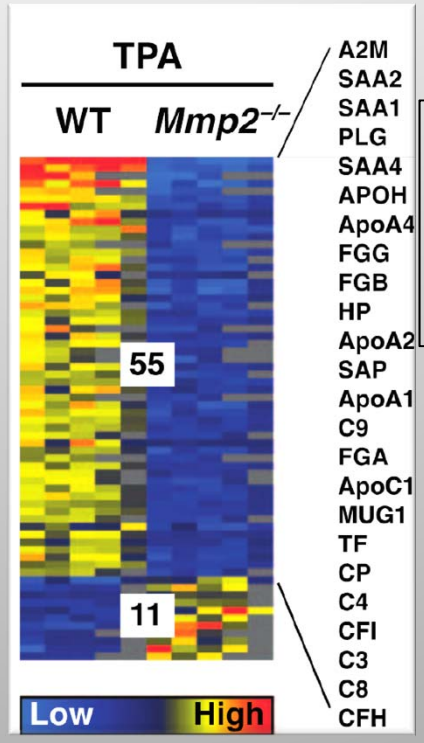
Hypothesis

WT inflammation:



Acute phase proteins
Complement
Coagulation
Proinflammatory S100
Myeloperoxidase (neutrophil influx)

WT vs KO inflammation:



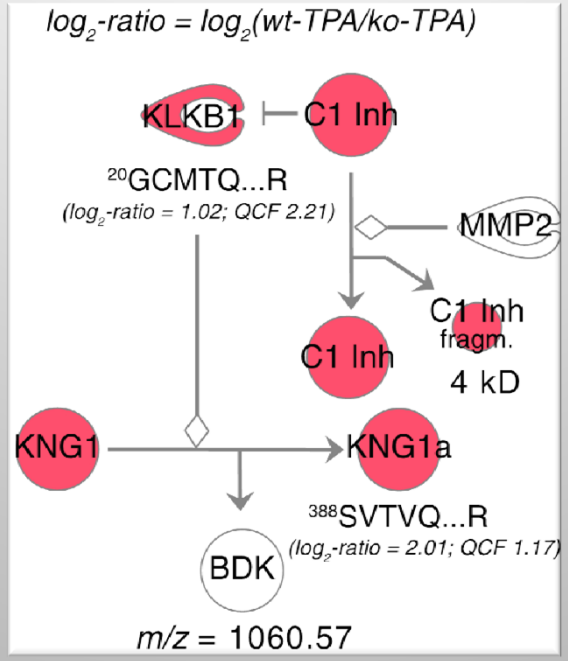
Decrease:
Reduction in the extent of exudation of acute-phase proteins, including complement factors and general plasma proteins

In $Mmp2^{-/-}$ mice there was less of an increase in vascular permeability in inflammation compared to that in wild-type mice, and hence, there was less exudation of serum and acute-phase proteins into the tissue.

Results

Digging the N-terminome

MMP2 and vascular permiability



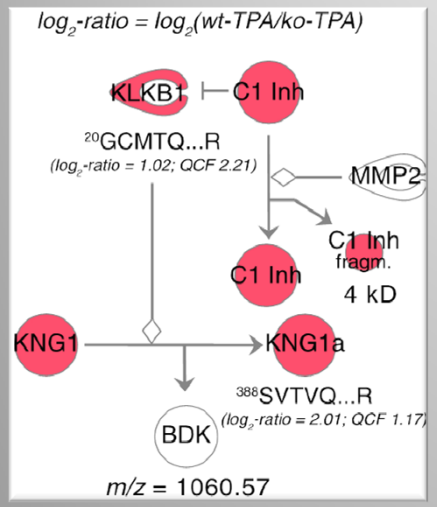
C1 inh – Serpin
KLKB1 – plasma kallikerin
KNG1 – kininogen
BDK – bradykinin (vascular permiability)

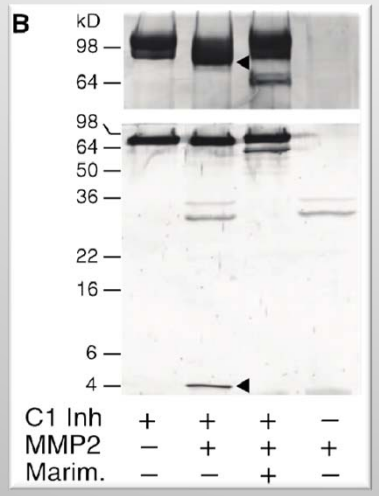
Results

MMP2 and vascular permiability

validation

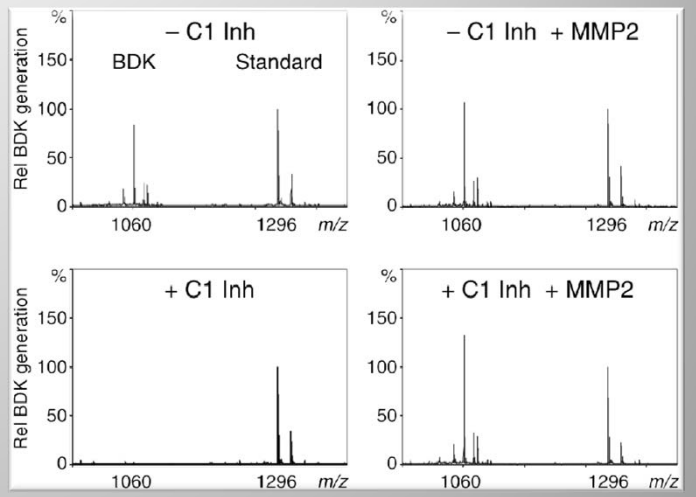
MS



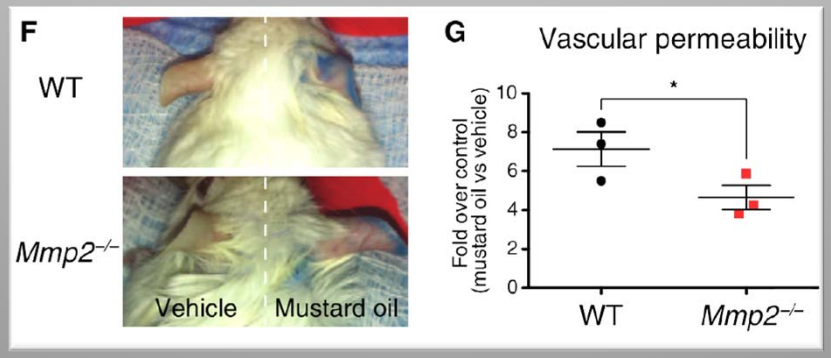


in vitro cleavage of C1 inh by MMP2

marimastat – MMP inhibitor



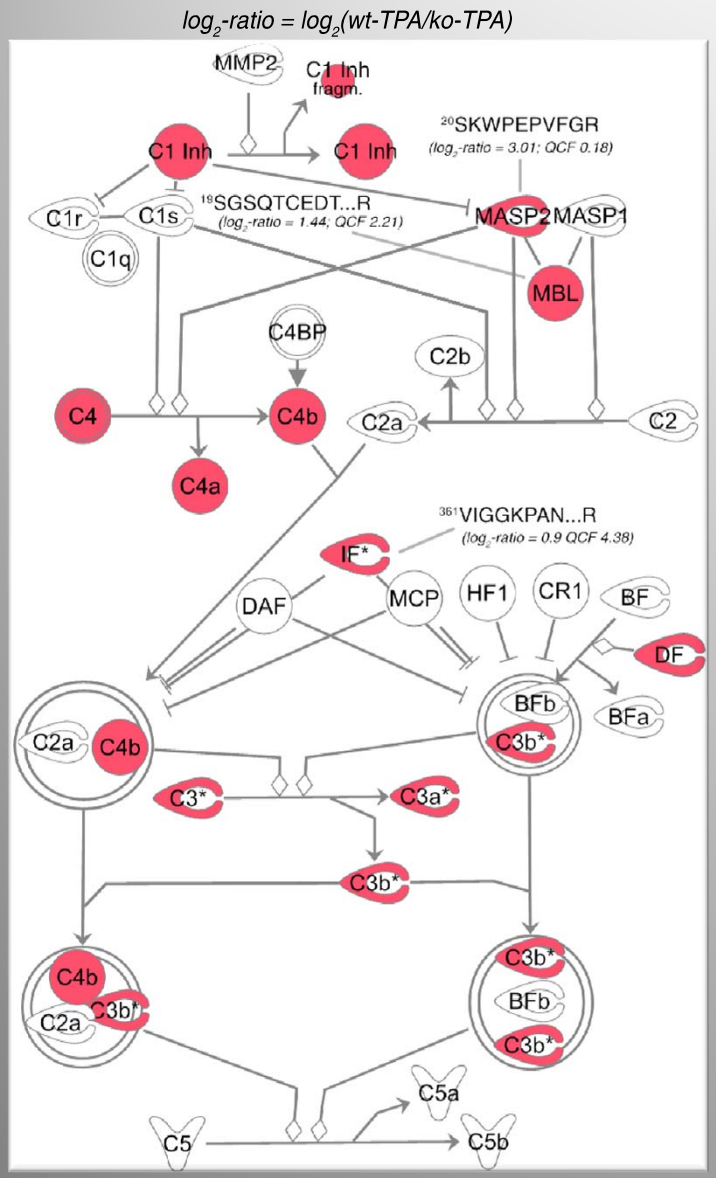
in vitro cleavage of KNG1 by KLKB



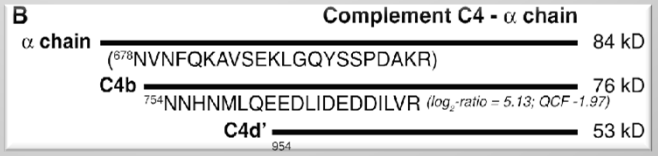
in vivo Miles assay using Evans Blue vascular permeability

Results

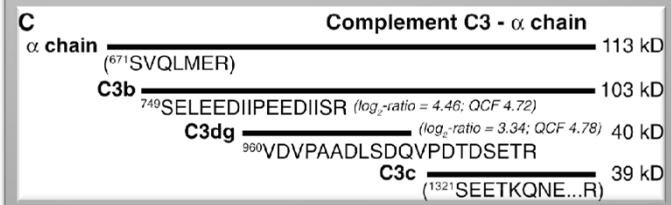
MMP2 and complement activation



MMP2 cleaves C1inh and enhances complement activation



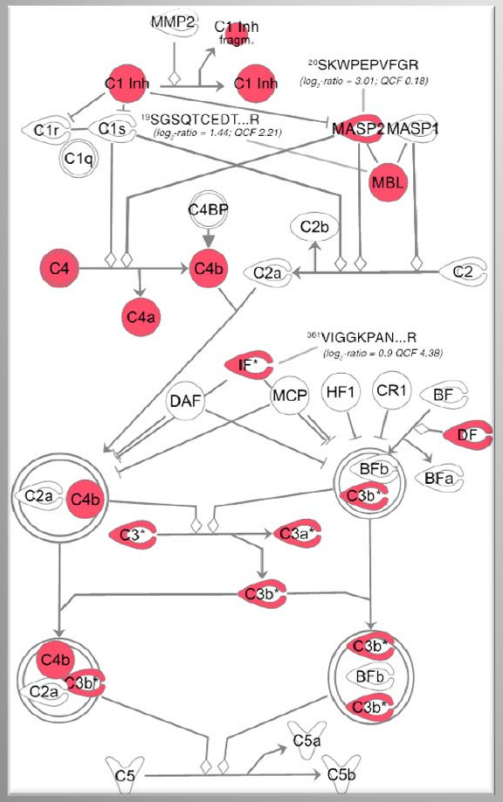
Complement factor I (IF) cleaves:
C4b to C4d'
C3b to C3dg
inactivating action

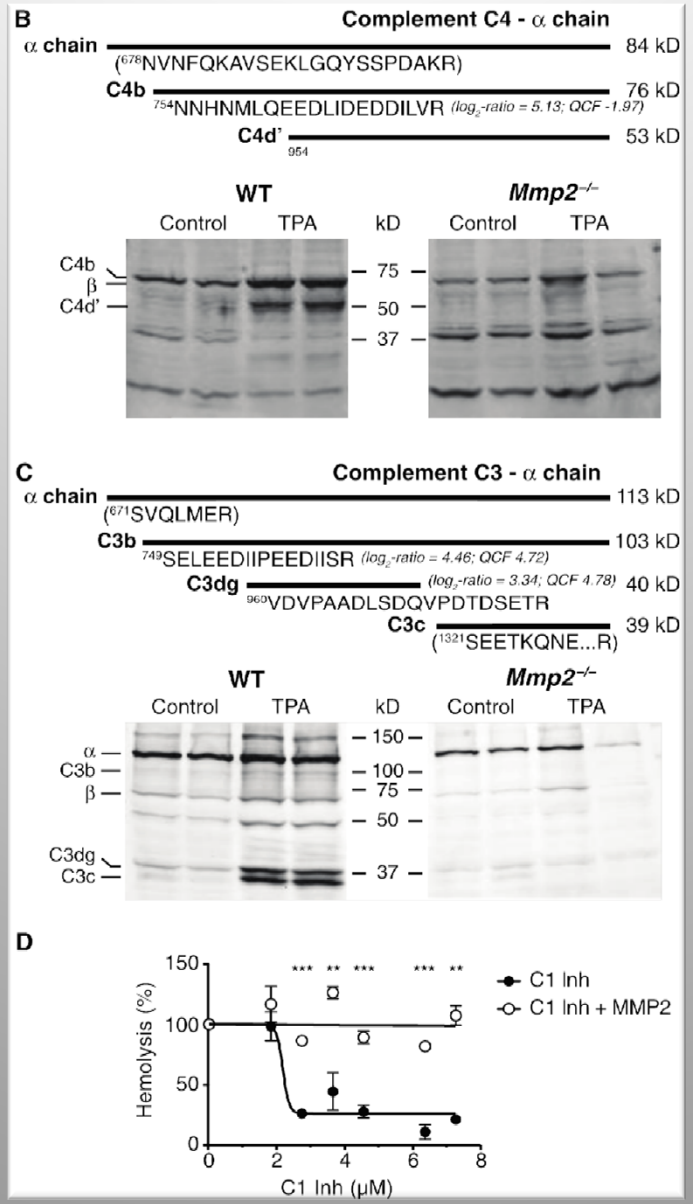


MMP KO mice – less C4b and C3b (and their inactivation products)

Results

MMP2 and complement activation, validation





ex vivo hemolysis
(membrane attack complex)

Discussion

1. Developed tool to read proteolytic signature of the inflamed tissue

2. Compared and quantified proteolytic signature of the inflamed tissue in WT and MMP2 KO
   - important and background proteolytic events

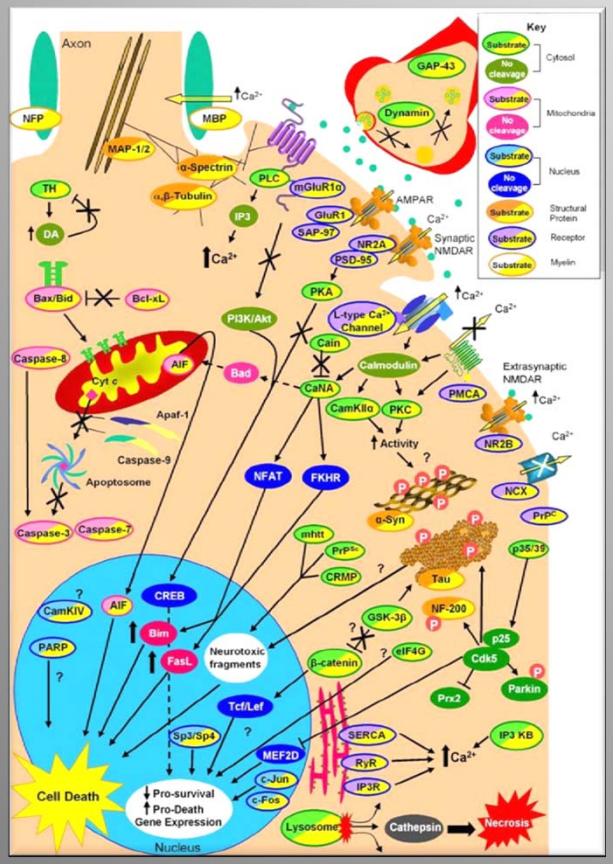
3. Identified proteins origin (newly imported in to the inflamed region)

4. Reconstructed the relevant proteolytic function of MMP2:
   - regulation of vascular permeability in inflammation (bradykinin release)
   - regulation of complement activation (C1 complex formation)

Possible implication

Prion Pathogenesis Is Faithfully Reproduced in Cerebellar Organotypic Slice Cultures.
Falsig J, Sonati T, Herrmann US, Saban D, Li B, Arroyo K, Ballmer B, Liberski PP, Aguzzi A.
PLoS Pathog. 2012

"prion neurotoxicity is calpain-dependent but caspase-independent in CGCs"



fodrin cleavage and…?

Vosler PS, Mol Neurobiol. 2008