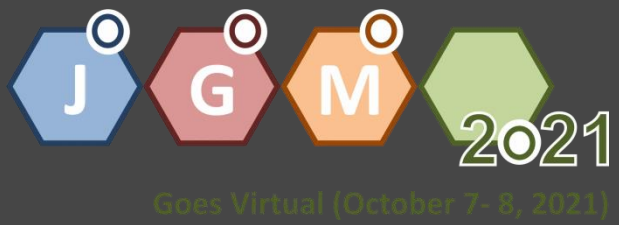
Joint Glycobiology Meeting - 2021



Bikunin: a biomarker of proteoglycan biosynthesis and Golgi homeostasis defects

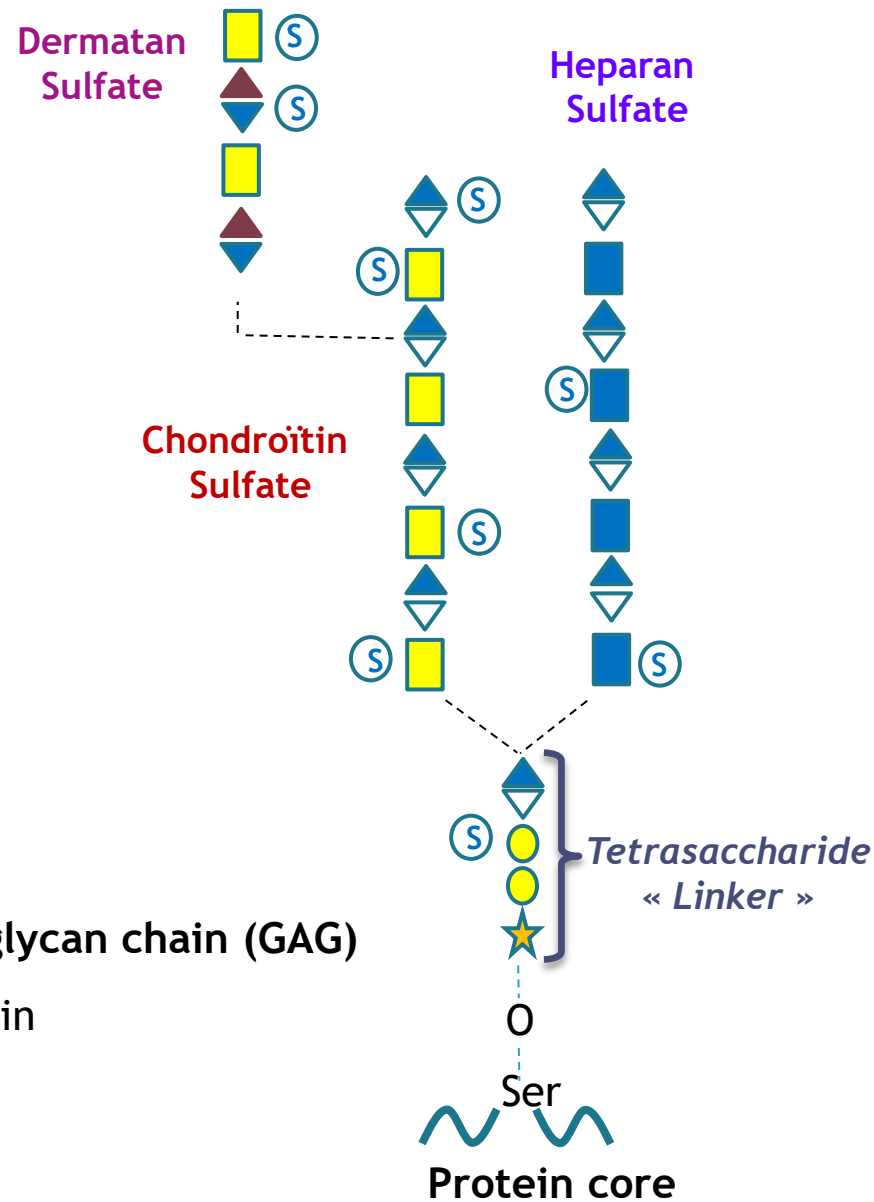
Dr Samra OUARAS LOUNIS

Walid Haouari
François Foulquier
Isabelle Cantaloube
Valérie Cormier-Daire
Christian Poüs
Arnaud Bruneel

INSERM UMR1193, Université Paris-Saclay, Faculté de Pharmacie
Châtenay Malabry, France

Proteoglycans

- Galactose
- Glucuronic acid
- N-acetylglucosamine
- Sulfate
- Xylose
- Iduronic acid
- N-acetylgalactosamine

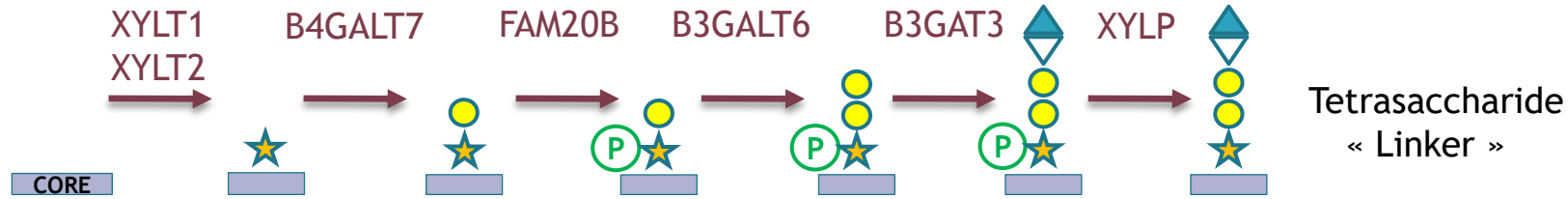


- **PG = core protein + Glycosaminoglycan chain (GAG)**
- ECMs of bones, joints, cartilage, skin
- Immune response

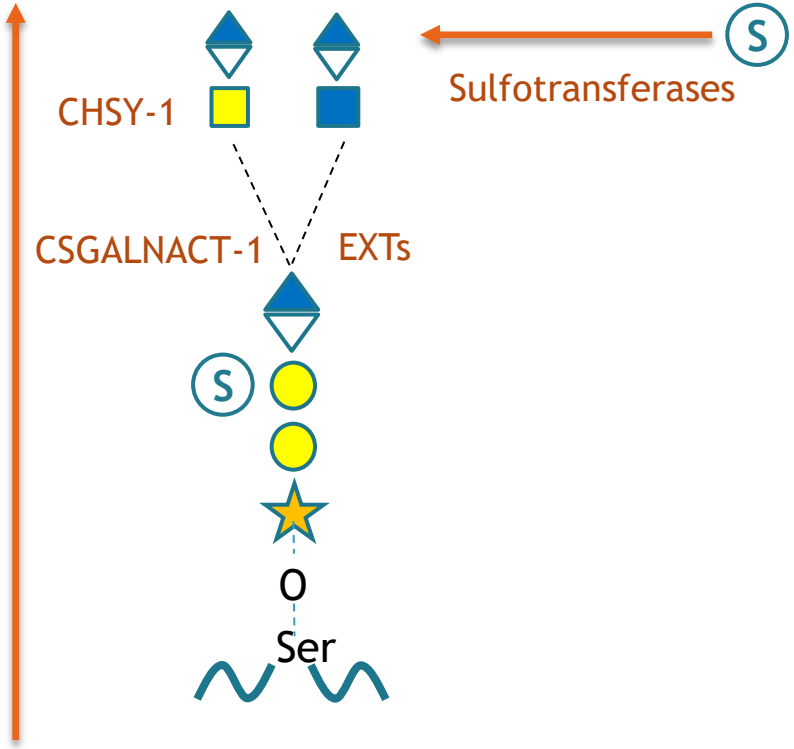
Inherited PG metabolic diseases

Direct PG biosynthesis defects

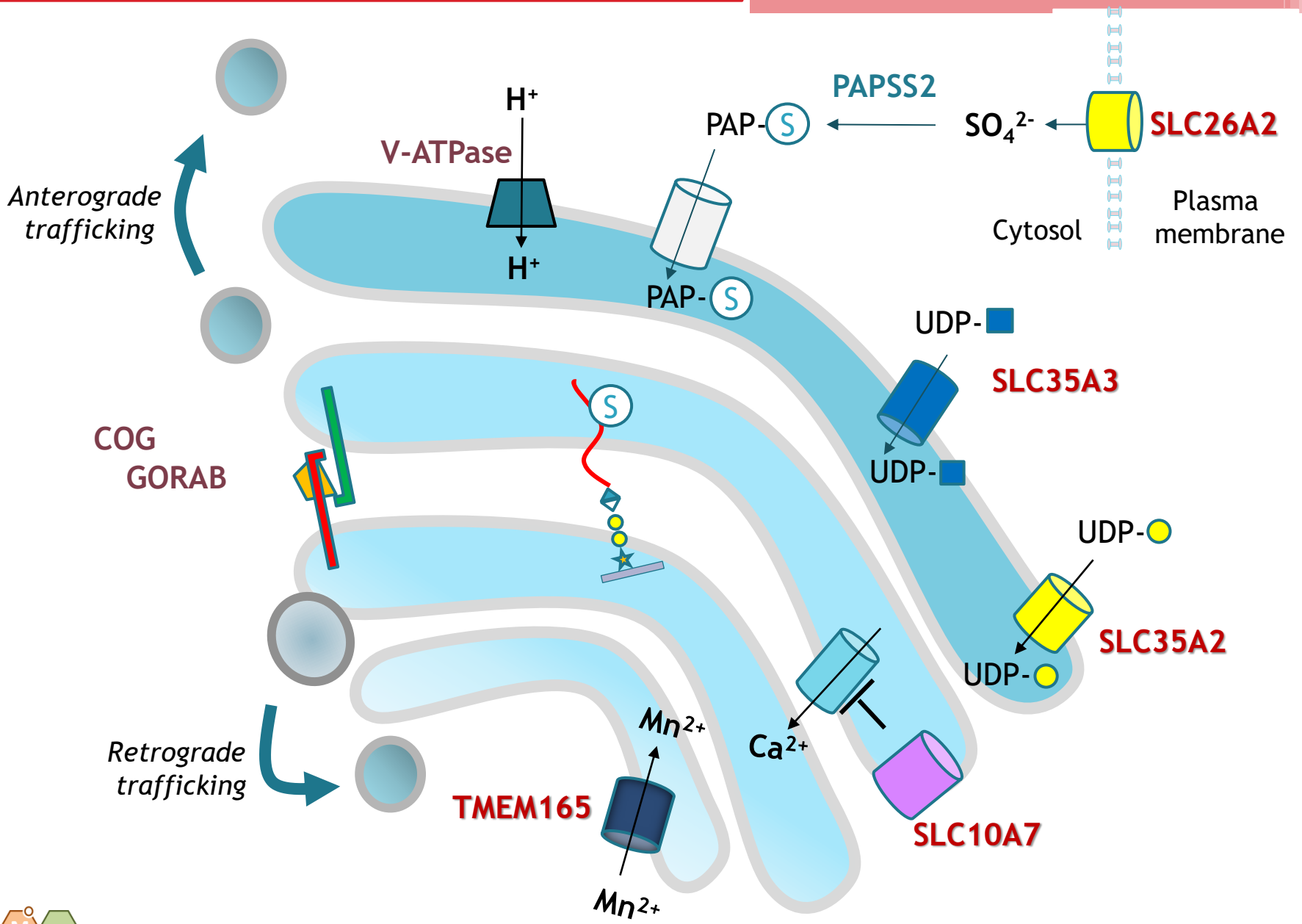
Linkeropathies



Elongation and sulfation defects



Indirect PG biosynthesis defects



Clinics

Osteoarticular defects

- Skeletal dysplasia
- Short stature, hand deformities
- Multiple fractures
- Joint dislocations and hyperlaxity

Unspecific symptoms

- Intellectual disabilities
- Skin, ocular, cardiac defects
- Deafness

*Leoni and al., AJMG, **2021***

*Sasarman et al., JIMD, **2016***

Current diagnosis strategy

Genetics

Gene sequencing

- Gene panels
- Whole exome/genome sequencing

Research

Patient fibroblasts

- Labelled substrate incorporation for PG and GAG quantification
- Functional studies

Blood and urine

- GAG levels and sulfation by HPLC/MS following chondroitinase/heparitinase

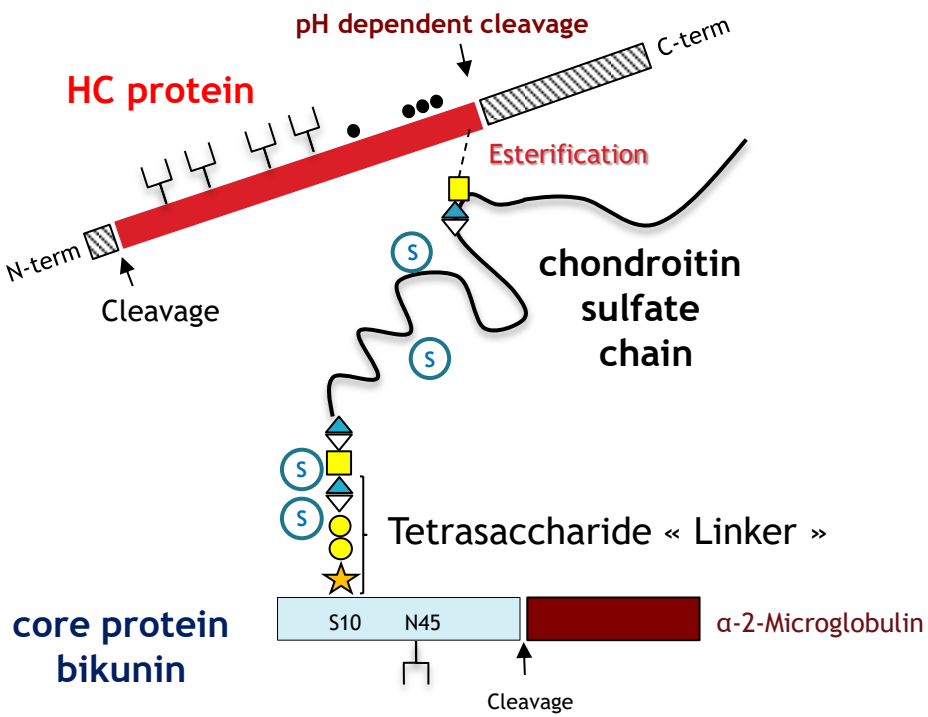
Lack of convenient routine blood biomarkers

Bikunin, an original circulating proteoglycan

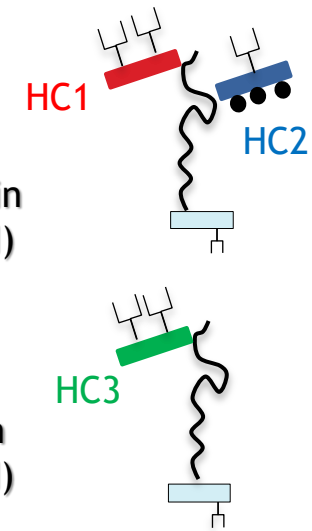
….a potential biomarker

- Xylose
- Galactose
- Glucuronic acid
- N-Acetylgalactosamine
- O-glycosylation
- N-glycosylation
- Sulfation

✓ Plasma protein
✓ Hepatocyte biosynthesis



Heavy forms
90-98%



Inter α trypsin Inhibitor (ITI)

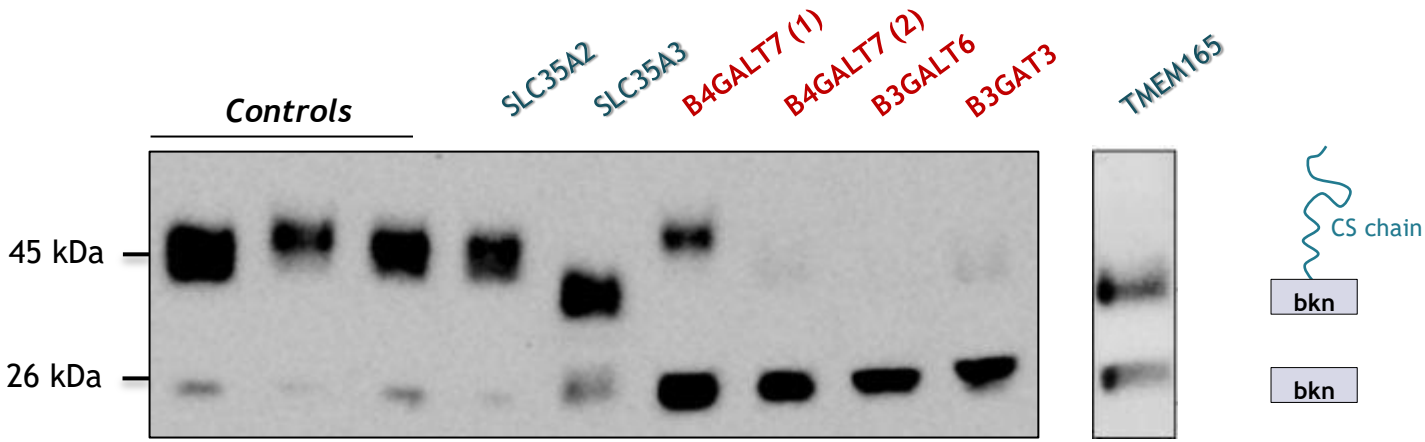
Pro-α trypsin Inhibitor (Pαl)

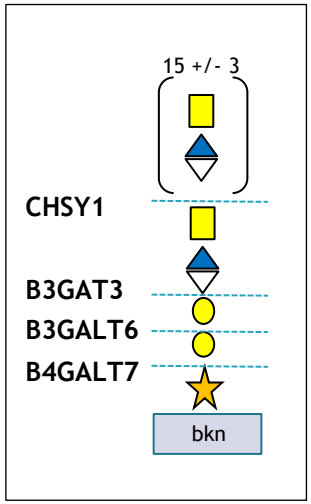
Light forms
2-10%

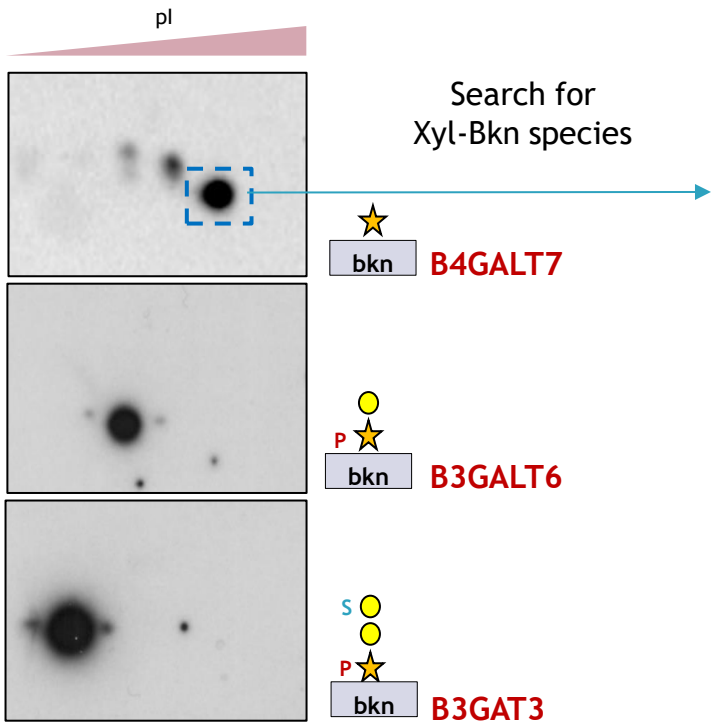
Urinary trypsin Inhibitor (UTI) Bkn-CS

Free core bikunin

Bikunin analyses in inherited PG defects



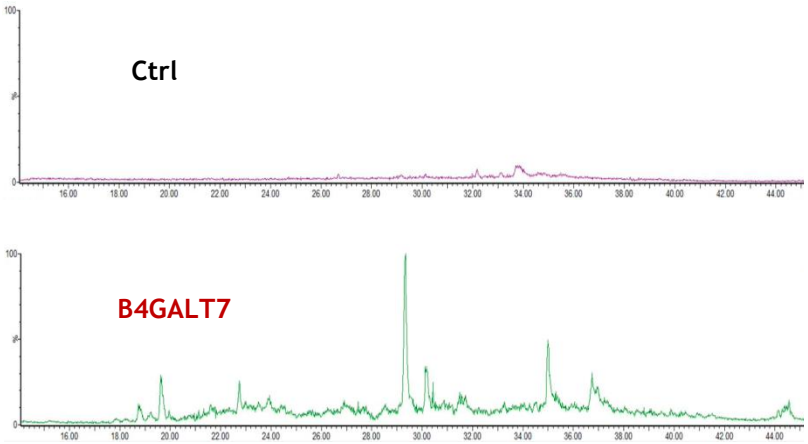




Search for
Xyl-Bkn species

HPLC/MS analysis

m/z 1131.06

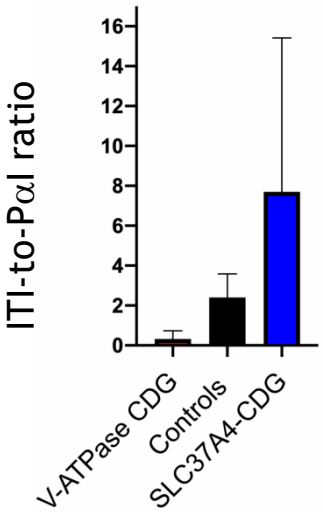


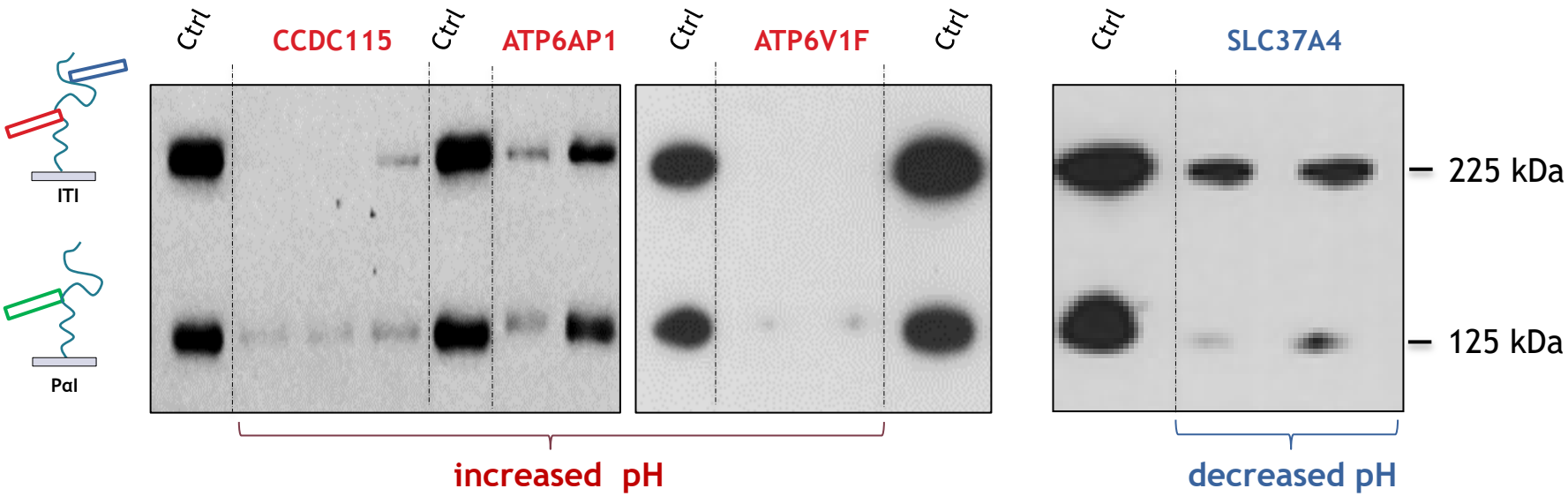
Haouari et al., JIMD 2020.

Bikunin in CDG with impaired Golgi pH homeostasis

Hallmarks of CDG with impaired V-ATPase

- Decreased levels of the heavy forms
- pH-dependent esterification perturbed
- Inversion of the ITI/Pαl ratio

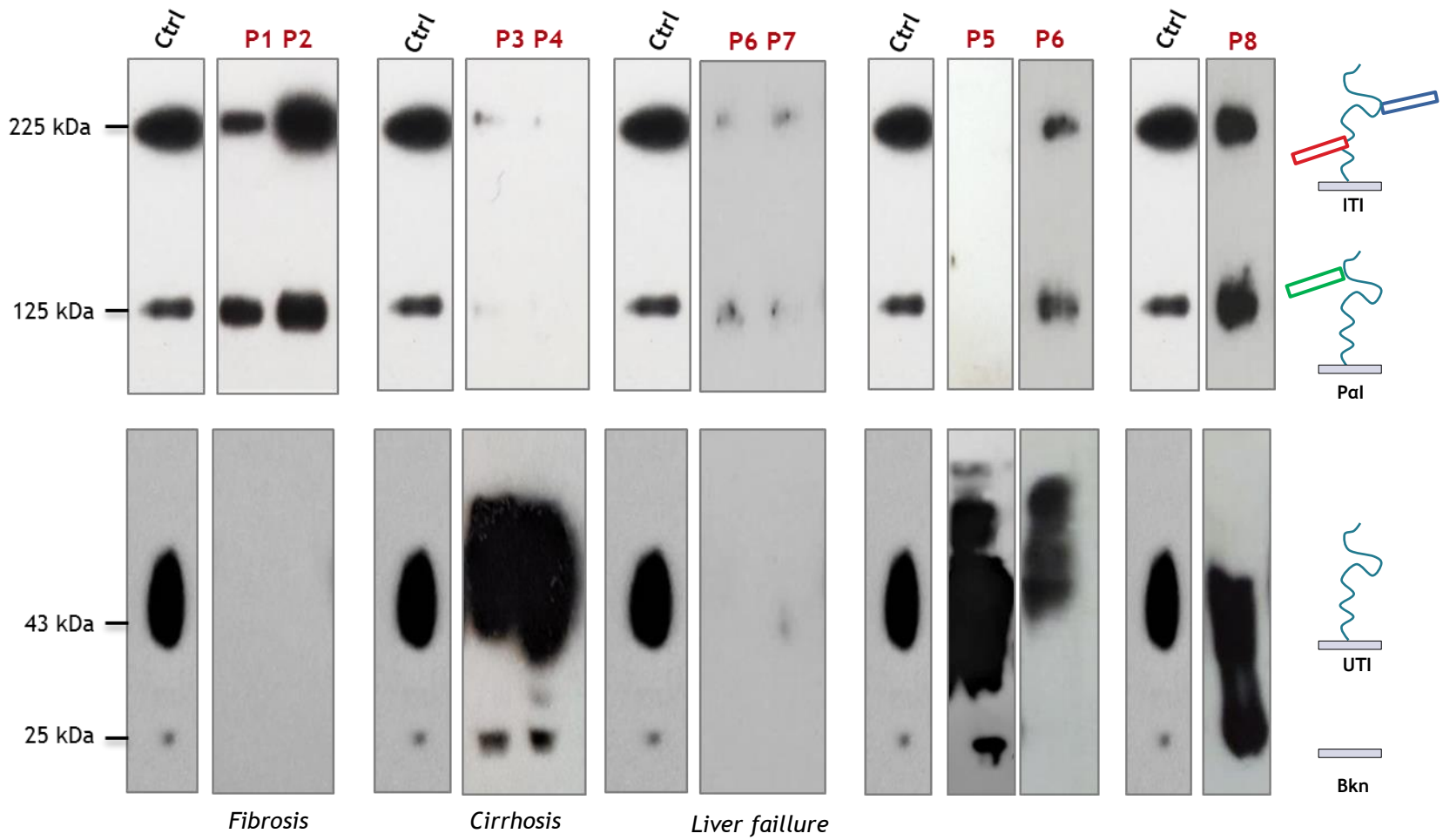




Haouari et al., JIMD 2020.

Bikunin isoforms in acquired diseases

Bikunin isoforms in acquired diseases



Liver diseases | Sepsis | Cancer

Effect of variation in extracellular pH

HepG2

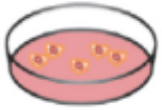
Hep3B

HuH7

HHL-16

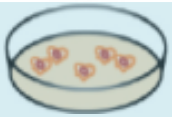
hepatoma or immortalized hepatocytes

Control Medium



Opti-MEM
(without FCS and thus without bovine Bkn)

Low pH Medium



Opti-MEM+ (2-to-8 mM) NaHCO3

High pH Medium

Opti-MEM+ (20-to-70 mM) NaHCO3

72-h culture

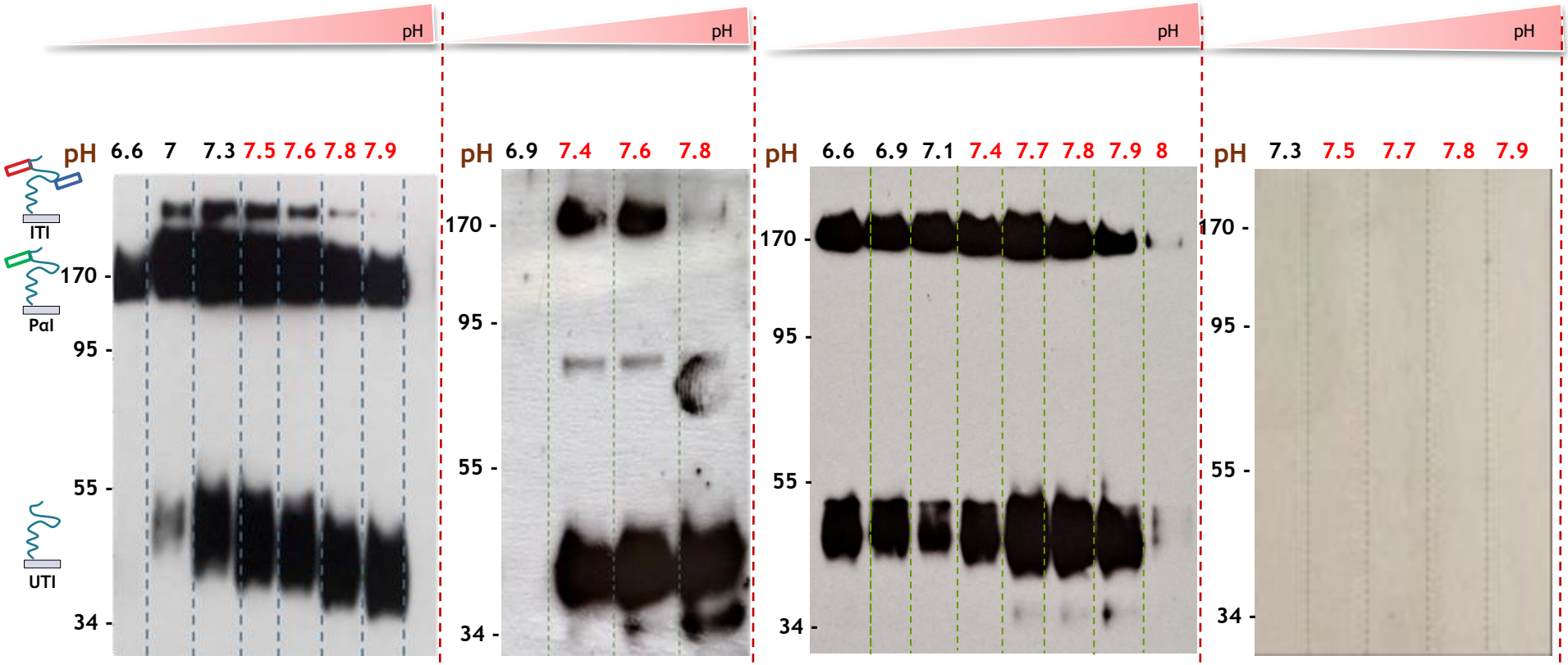
Recovery of supernatants and cell lysates

WB analysis

Effect of variation in extracellular pH

EXTRACELLULAR pH VARIATIONS

Culture supernatants



N=4 | N=3 | N=3 | N=2

HepG2
Human hepatocellular carcinoma cells

Hep3B
Human hepatoma cell line

HuH7
Human hepatocellular carcinoma cells

HHL16
Immortalized hepatic cell line

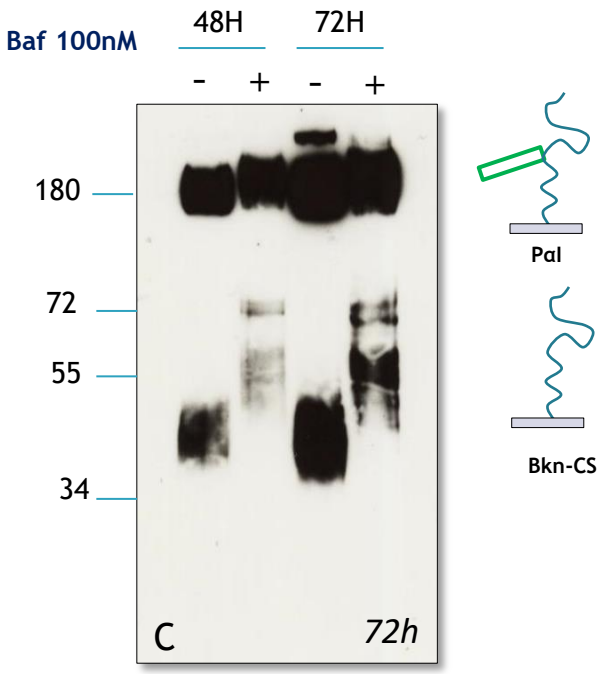
HepG2 culture in the presence of Bafilomycin

INTRA-GOLGI ALKALIZATION

Bafilomycin (Baf)

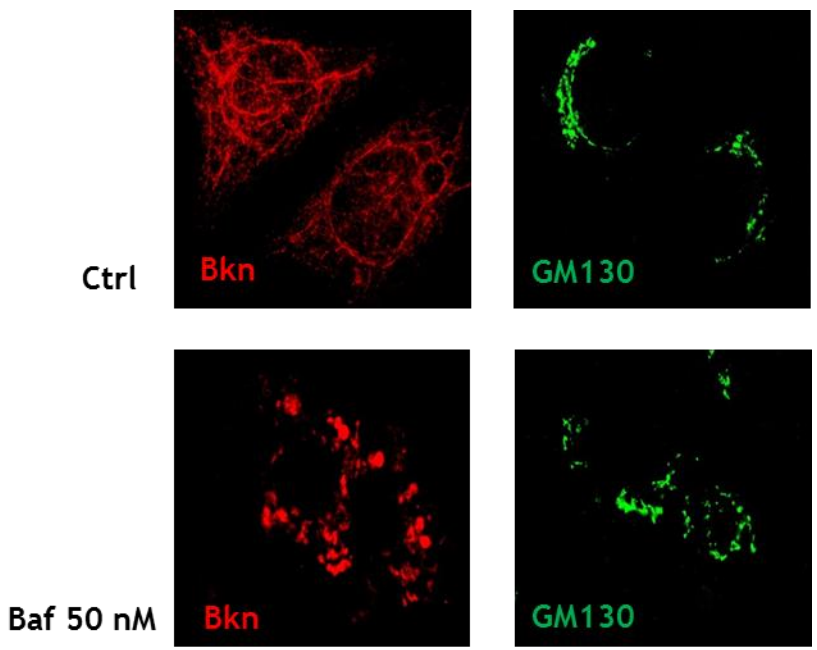
- V-ATPase inhibitor
- Impaired protein glycosylation

HepG2 *(cellular supernatant)*



Increased molecular weight of Bkn-CS and Pαl after treatment with bafilomycin.

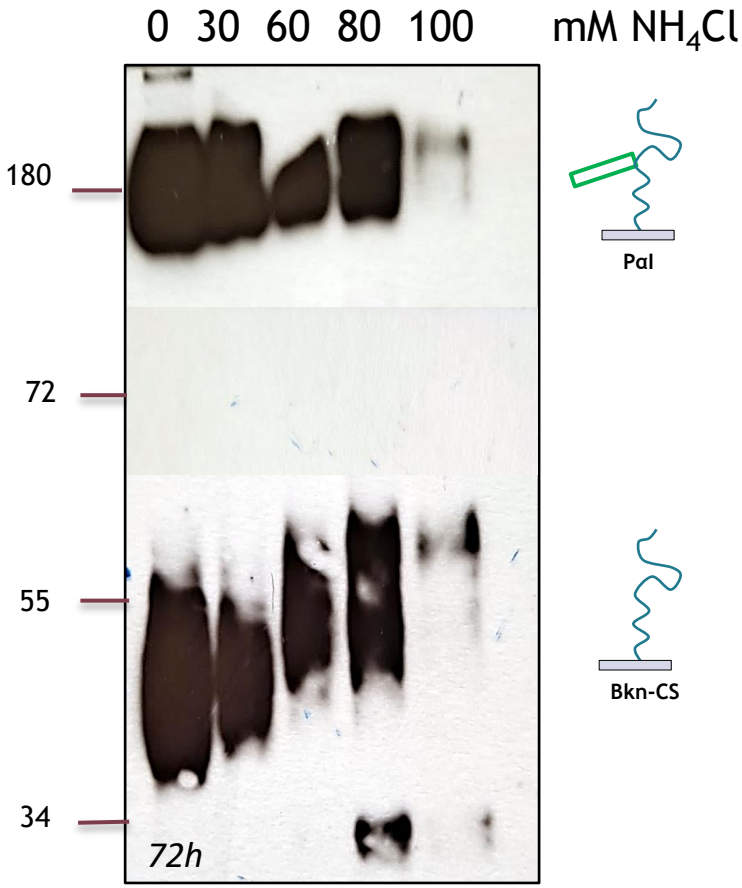
HepG2 *(intracellular)*



HepG2 culture in the presence of NH_4Cl

INTRA-GOLGI ALKALIZATION

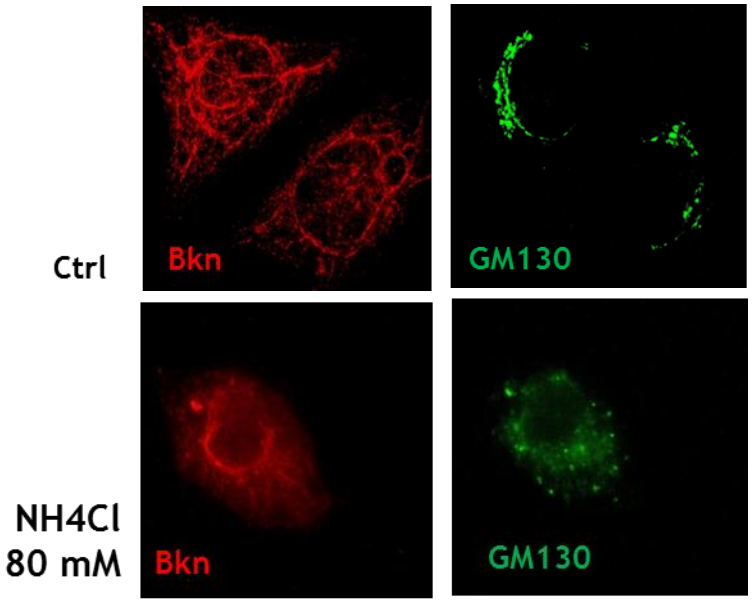
HepG2 *(cellular supernatant)*



NH_4Cl

- Weak base that reversibly increase the pH of intracellular acidic compartments.

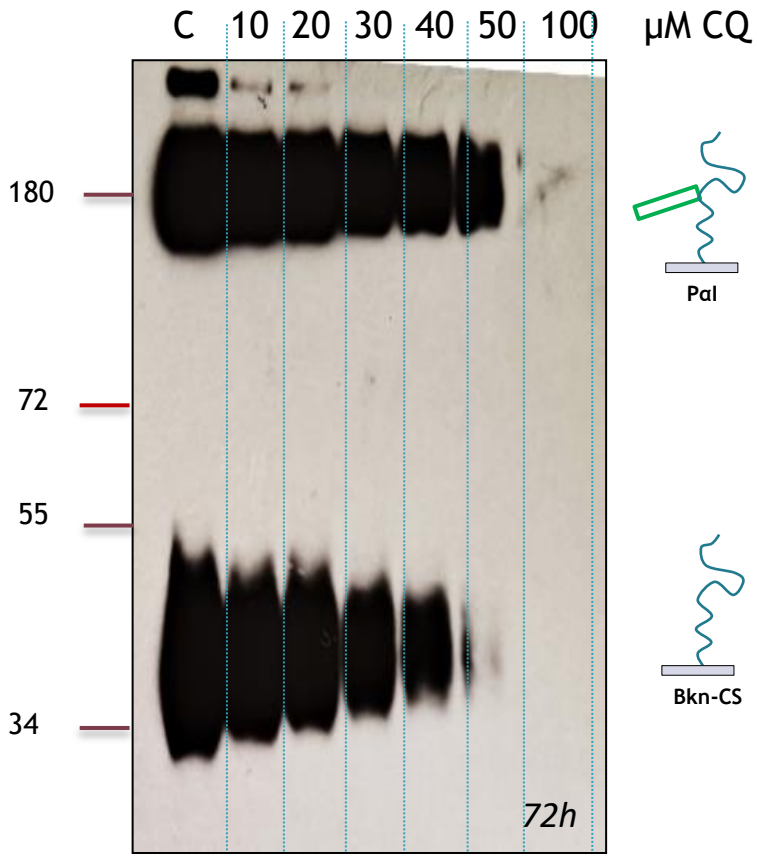
HepG2 *(intracellular)*



HepG2 culture in the presence of Chloroquine

INTRA-GOLGI ALKALIZATION

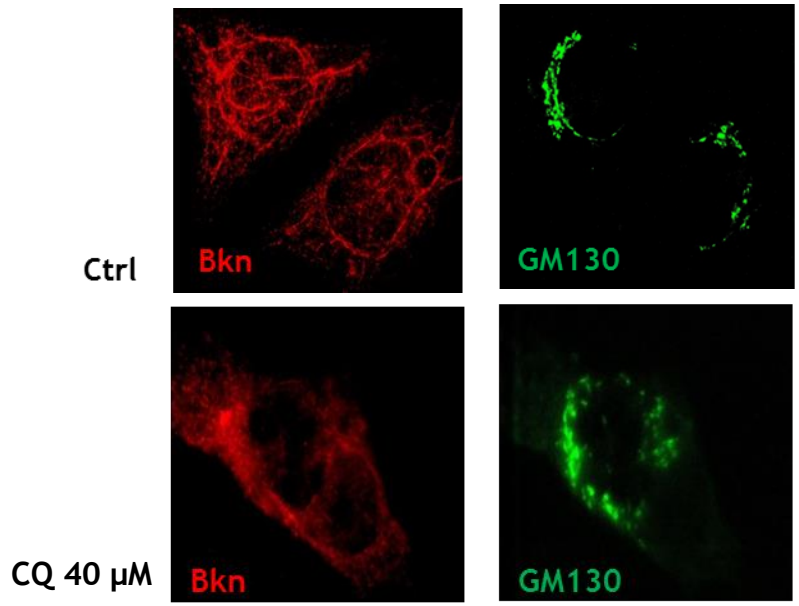
HepG2 *(cellular supernatant)*



Chloroquine (CQ)

- Weak base that reversibly increase the pH of intracellular acidic compartment

HepG2 *(intracellular)*



Conclusions

- Bikunin: an versatile biomarker for identifying GAG biosynthesis and Golgi homeostasis defects.
- Importance of Golgi pH regulation on qualitative and quantitative aspects of Bikunin biosynthesis.
- Complete these studies with:
  - intra-Golgi pH measurements
  - MS analysis of CS chain → activity of the enzymes involved (elongation and/or sulfation)
  - Expression and dynamics of fluorescent Bikunin
- Better connect in vitro observations to pathophysiology in patients

Acknowledgments

Coordination

Arnaud BRUNEEL

Christian POÜS

Walid HAOUARI

Collaborations

François FOULQUIER

François FENAILLE

Bobby NG, Hudson FREEZE

Valérie CORMIER-DAIRE

INSERM U1193
"Pathophysiology of liver diseases"

Arnaud BRUNEEL

Christian POÜS

Walid HAOUARI

Isabelle CANTALOUBE

Sara MERABET

Antoine PILON

Anita BAILLET

Béatrice BENOIT

Daniel PERDIZ

Elise JACQUIN

Najet CHAREF

Bruno BAUDIN

Alexandre RAYNORD

Ameetha RATIER