Differential MMP-14 Targeting by Biglycan, Decorin, Fibromodulin and Lumican Unraveled by In Silico Approach

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FLORENCE
28-30 SEPTEMBER 2022

Differential MMP-14 Targeting by Biglycan, Decorin, Fibromodulin and Lumican Unraveled by *In Silico* Approach

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**Extracellular Matrix and Cell Dynamics (MEDyC), CNRS UMR/URCA N° 7369,
Faculté de Médecine, Reims, France
Head: Pr L. Martiny**

Lumican

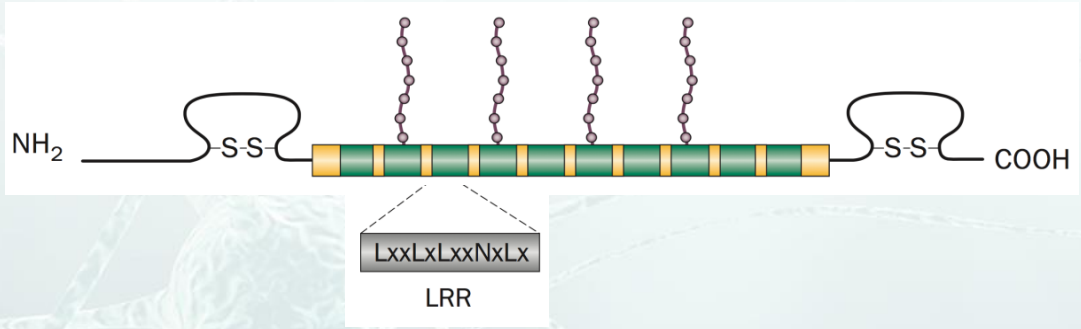
- ✓ **small leucine-rich proteoglycan (SLRP)**
- ✓ **abundant within tumor reactive stroma**

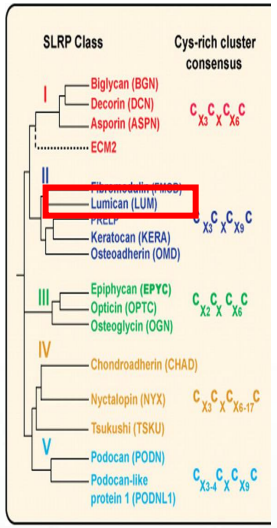
In melanoma:

- ↘ lumican expression = more infiltrative disease *Brézillon et al., Clin Exp Dermatol 2007*
- promotes cell adhesion and inhibits cell migration
  D'Onofrio et al., Biochem Biophys Res Commun 2008 ;
  Brézillon et al., Cancer Lett 2009 ; Zeltz et al., Exp Cell Res 2010 ; Stasiak at al., PLoS One 2016;
  Jeanne et al., Scientific reports, 2017;
  Brézillon et al., Frontiers in Cell and developmental Biology, 2020; Dauvé et al., Cancers, 2021
- angiostatic properties *Brézillon et al., J Physiol Pharmacol, 2009*

In ovarian cancer:

Nizet et al., Cancers, 2021





[Biological functions of the small leucine-rich proteoglycans: from genetics to signal transduction.](#)
Adapted from Schaefer L, Iozzo RV.J Biol Chem. 2008

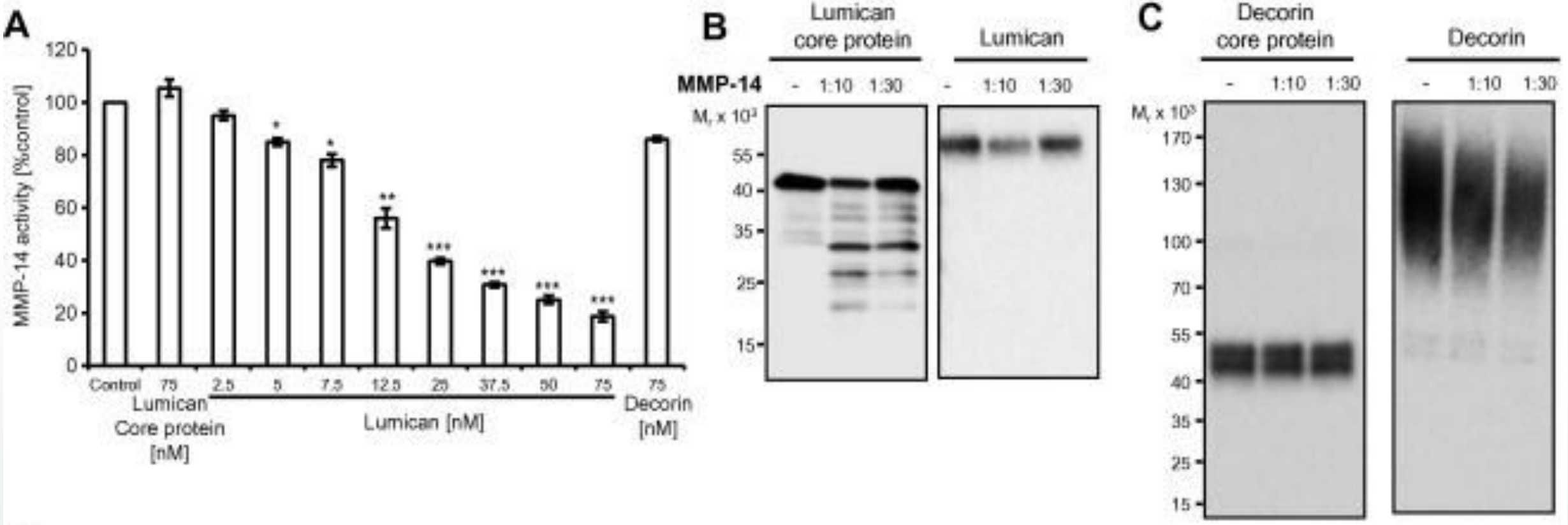
Kao *et al.*, 2006

Core protein
(338 AA, 37 kDa)

Glycoprotein (skin)
(57 kDa)
N-glycosylation sites:
88, 127, 160, 252

KSPG (cornea)
(70-170 kDa)

LUMICAN = KEY REGULATOR OF COLLAGEN FIBRILLOGENESIS



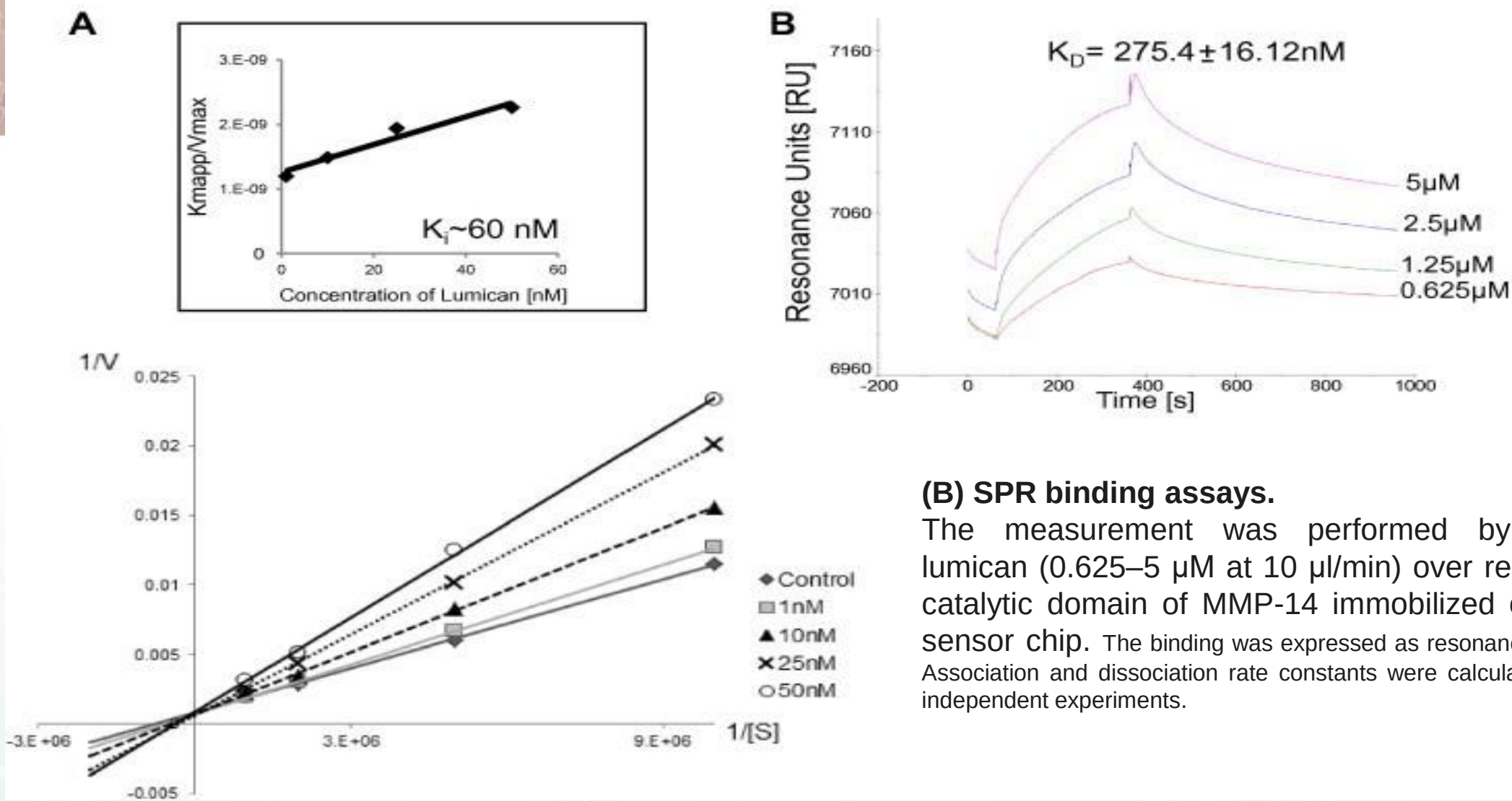
A) Glycosylated lumican is an inhibitor of MMP-14 activity but glycosylated decorin is a poor inhibitor.

The activity of the catalytic domain of MMP-14 (5 nM) pre-incubated 15 min before assay at 37 °C with lumican core protein (75 nM), or lumican (2.5–75 nM) or decorin (75 nM). The activity of MMP-14 was measured using fluorimetric SensoLyte® 520 MMP-14 Assay Kit.

B, C) Lumican core protein is a MMP-14 substrate but glycosylated lumican is not cleaved as decorin.

Degradation of lumican (B) or decorin (C) by MMP-14. One microgram of lumican core protein or entire molecule (B) and decorin core protein or entire molecule (C) was incubated in reaction buffer, with recombinant catalytic domain of MMP-14 at 37 °C for 17 h in the indicated molar ratio enzyme to SLRP protein. Products of enzymatic reaction were separated on SDS–PAGE electrophoresis and analyzed by Western blotting by anti-lumican (B) or anti-decorin (C) antibodies.

Pietraszek *et al*., FEBS Lett. 2014



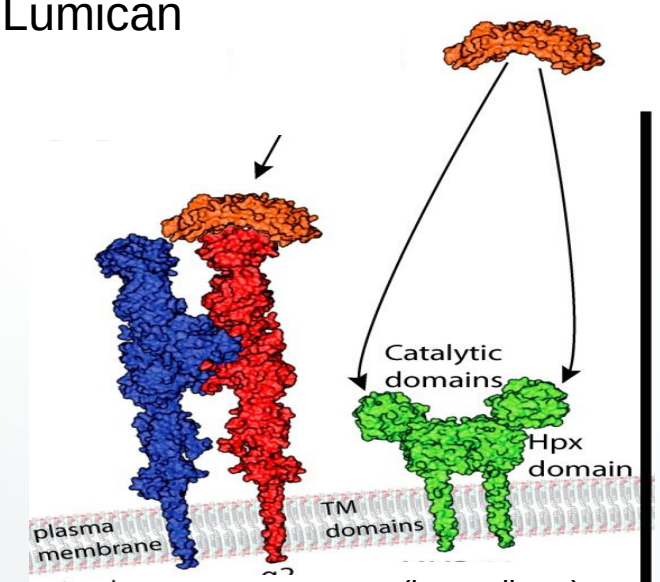
(B) SPR binding assays.
The measurement was performed by injecting lumican (0.625–5 μM at 10 μl/min) over recombinant catalytic domain of MMP-14 immobilized on a CM5 sensor chip. The binding was expressed as resonance units (RU). Association and dissociation rate constants were calculated from two independent experiments.

(A) Lumican is a competitive inhibitor of MMP-14 activity.
Increasing concentrations of substrate (0.01–1 μM) were added to a mixture of MMP-14 (5 nM) and lumican (1–50 nM). Fluorimetric measurements of enzymatic hydrolysis of the substrate was presented as a Lineweaver–Burk plot. K_i value was obtained from the linear regression plot of K_{Mapp}/V_{MAX} as a function of the inhibitor (insert). Similar results were obtained from two independent experiments. [S] – substrate concentrations (M); [V] – enzymatic reaction velocity (fluorescence units/second).

Pietraszek *et al*., FEBS Lett. 2014

Identification of lumican receptors

Lumican



Integrin (homodimer)

Apoptosis
Adhesion
Migration
Invasion
MET

Migration
Invasion
Metastasis
MET

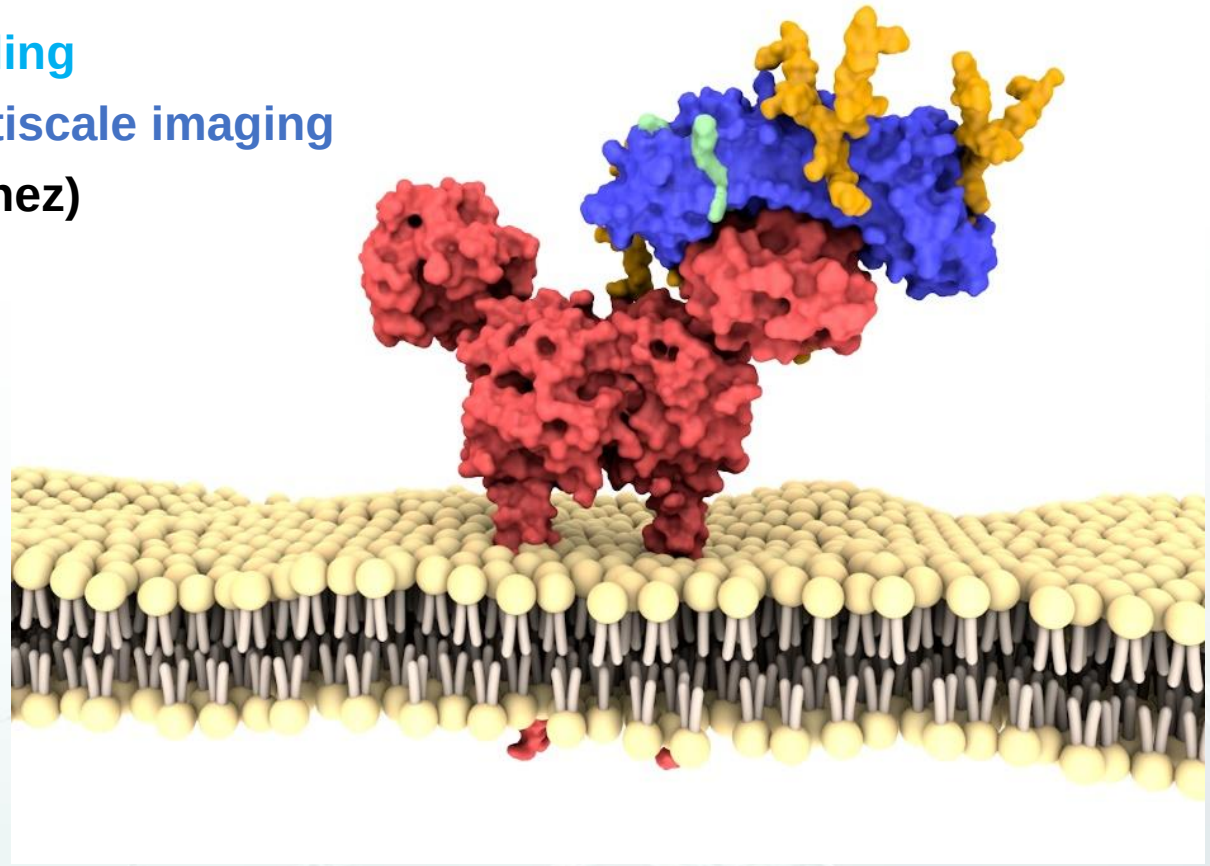
Zeltz et al., Exp. Cell Res., 2010

Pietraszek et al., FEBS Lett., 2014;
Pietraszek-Gremplewicz et al., Matrix Biology, 2019

→**Molecular Modeling**

Modeling and multiscale imaging

(S. Baud, M. Dauchez)



Human MMP-14 (dimer) interacting with N-glycosylated lumican

Karamanos et al., Chem. Rev., 2018

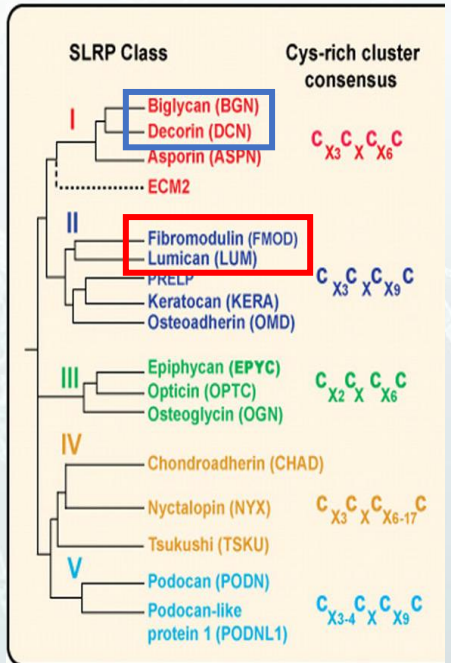
AIM

To characterize, by *in silico* 3D modeling, the structure and the dynamics of Biglycan (BGN), Decorin (DCN), Fibromodulin (FMOD) and Lumican (LUM), including their core protein and their specific polysaccharide chains

to assess the SLRP capacity

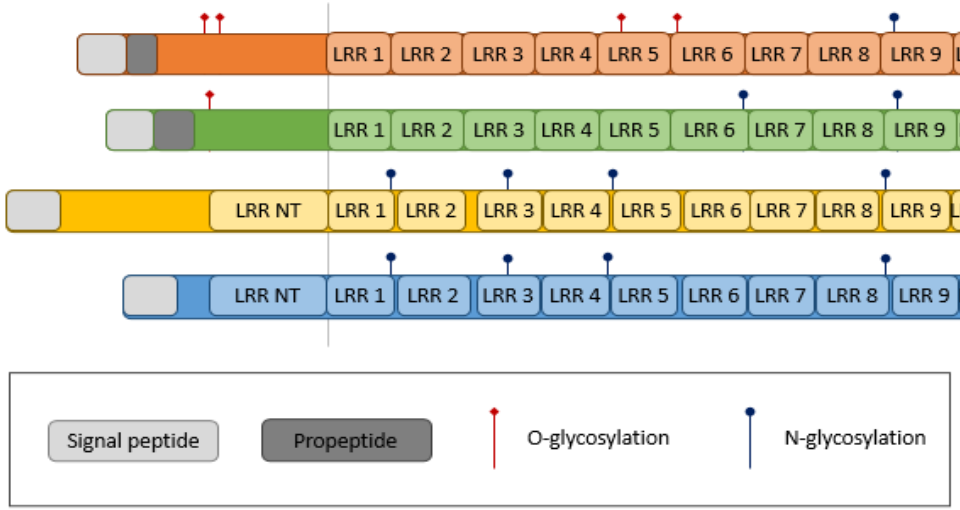
1) to regulate MMP-14 activity,

2) to be cleaved by MMP-14.

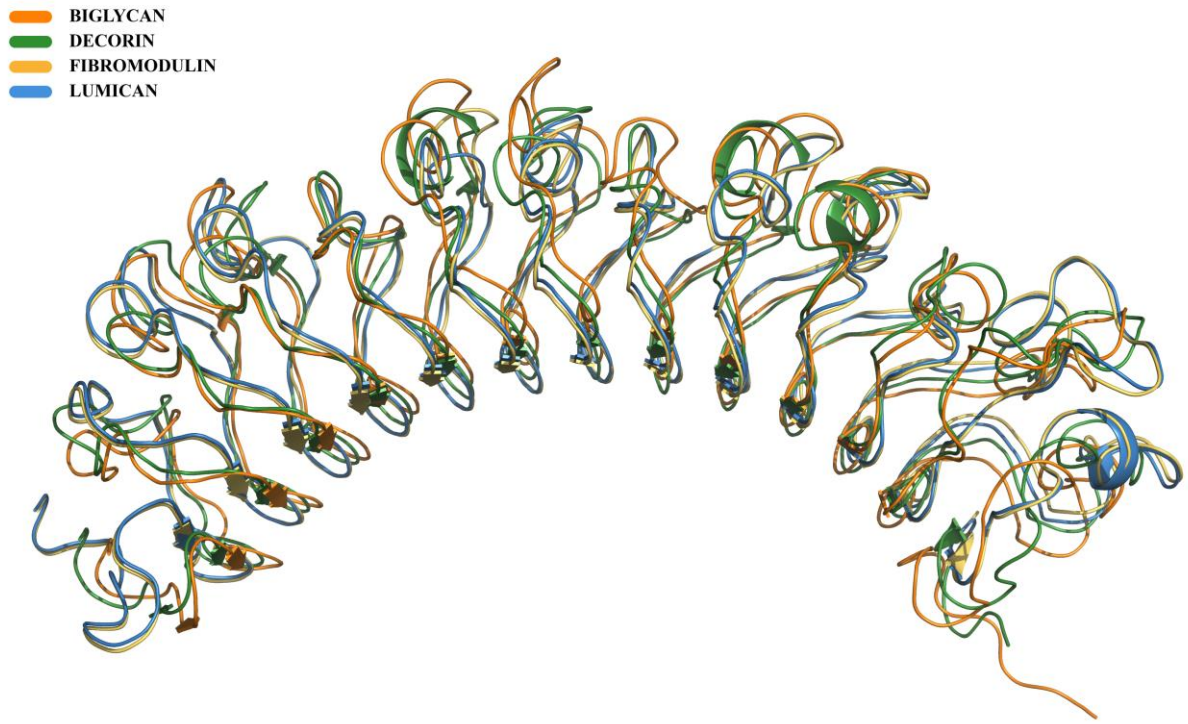


Biological functions of the small leucine-rich proteoglycans: from genetics to signal transduction.
Adapted from Schaefer L, Iozzo RV.J Biol Chem. 2008

Comparisons of the human biglycan (BGN), decorin (DCN), fibromodulin (FMOD), and lumican (LUM) core protein structures and post-translational modifications positions.



Biglycan O-glycosylation sites : 42, 47, 180, 198
Biglycan N-glycosylation sites : 270, 311
Decorin O-glycosylation site : 34
Decorin N-glycosylation sites : 211, 262, 303
Fibromodulin N-glycosylation sites : 127, 166, 201, 291, 341
Lumican N-glycosylation sites : 88, 127, 160, 252

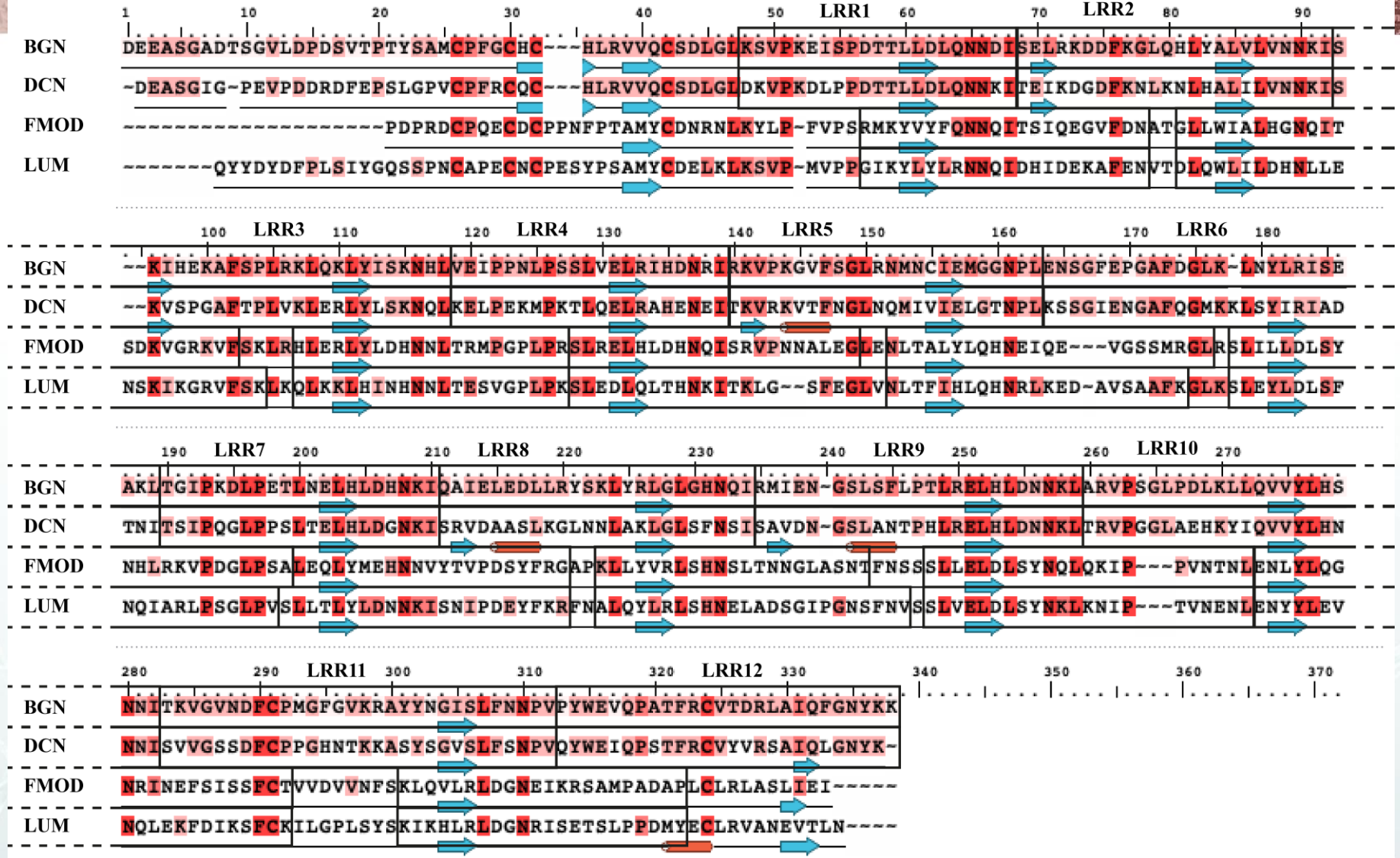


(A) Schematic comparison of the LRR sequences of BGN, DCN, FMOD, and LUM from LRR1 to LRR12 and positions of their O- and N-glycosylation sites. Signal peptide and propeptide are depicted. The locations of the LRR and glycosylation sites were extracted from the UniProt server using the sequence references, UniProtKB AC: Biglycan (P21810), Decorin (P07585), Fibromodulin (Q06828), Lumican (P51884).

(B) Structural alignment of the four SLRP structures.

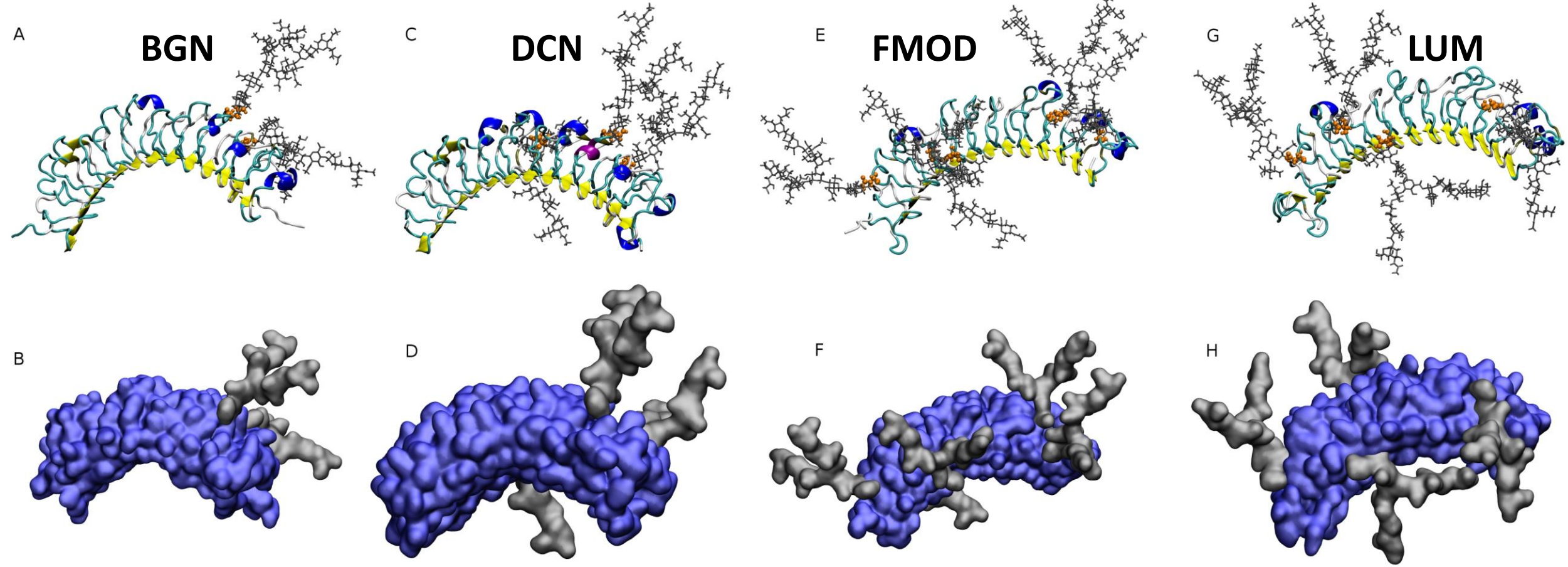
Rivet *et al*., submitted to AJP Cell Phys.

Comparison of the human biglycan (BGN), decorin (DCN), fibromodulin (FMOD), and lumican (LUM) core protein structures: Dual presentation of the sequence alignment and the local secondary structure alignment.



(C) Dual presentation of the sequence alignment and the local secondary structure alignment. Sequence conservation is highlighted by colored letters: pink (identity for two out of four sequences), dark red (identity for all four sequences). Elements of the local secondary structure are depicted using blue arrows (β-sheets) and red cylinders (α-helices). LRR position is indicated as rectangular boxes.

Rivet *et al.*, submitted to AJP Cell Phys.



Secondary structures and N-glycosylation positions on human biglycan (BGN, A,B), decorin (DCN, C,D), fibromodulin (FMOD, E,F) and lumican (LUM, G,H).

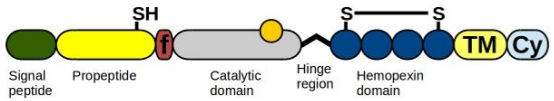
For each human SLRP, two types of representations are shown: a **cartoon representation of the backbone** (A, C, E and G) and **a surface representation** that also considers the occupancy of the side chains (B, D, F and H).

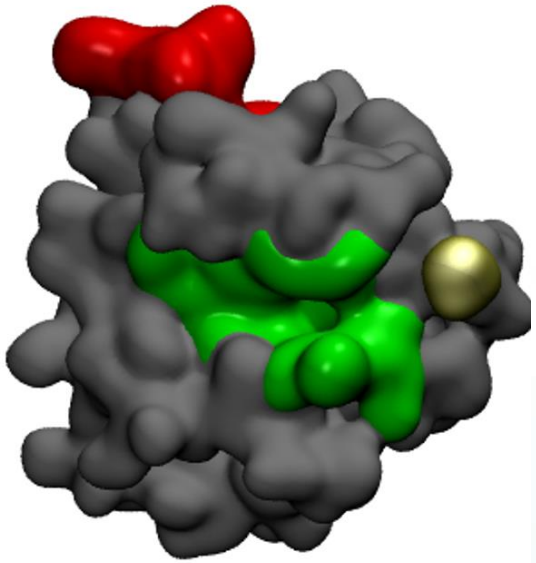
The cartoon representations are colored according to the secondary structure of the core proteins and the residues bearing the N-glycosylations are displayed using orange Van der Waals motifs. Bi-antennary glycosylated chains are modeled with gray licorice (A, C, E and G) or gray surfaces (B, D, F and H).

Human Fibromodulin Q06828 (PDB ID 5MX0).

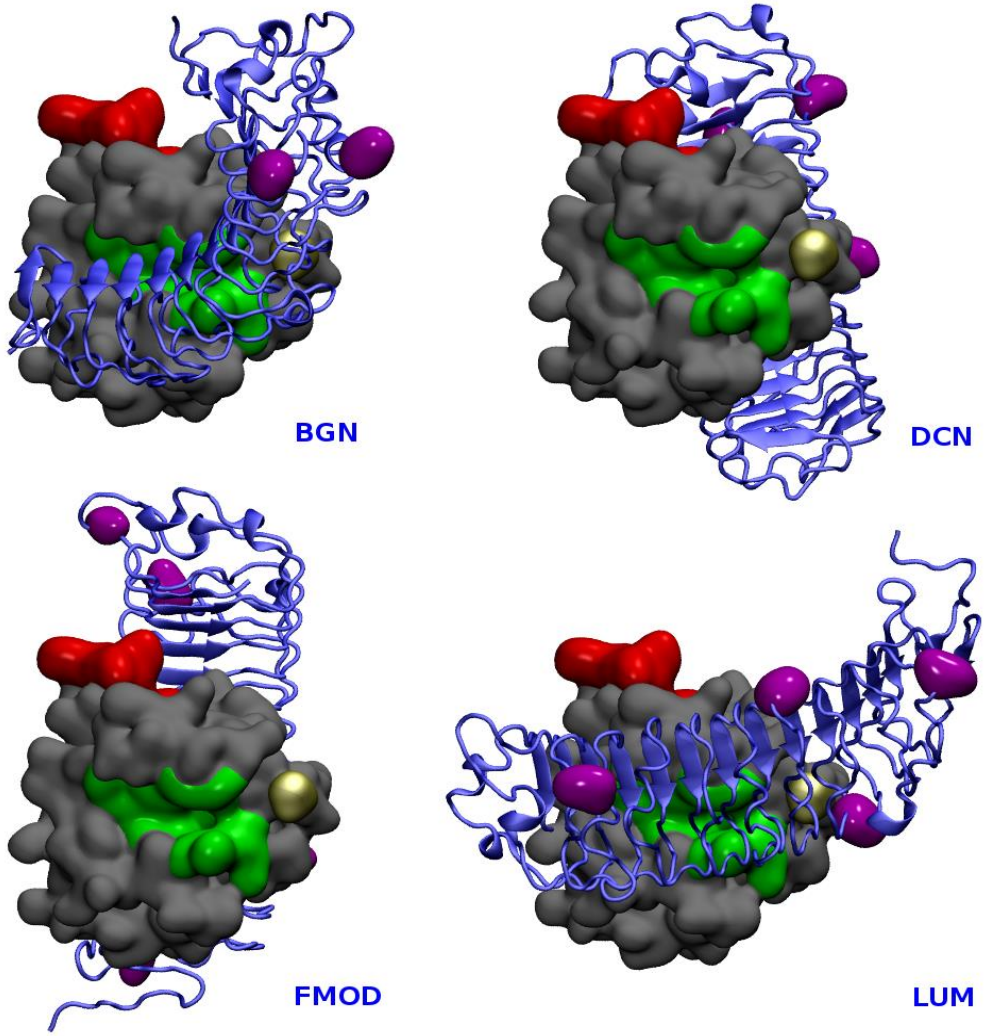
Rivet *et al*., submitted to AJP Cell Phys.

A



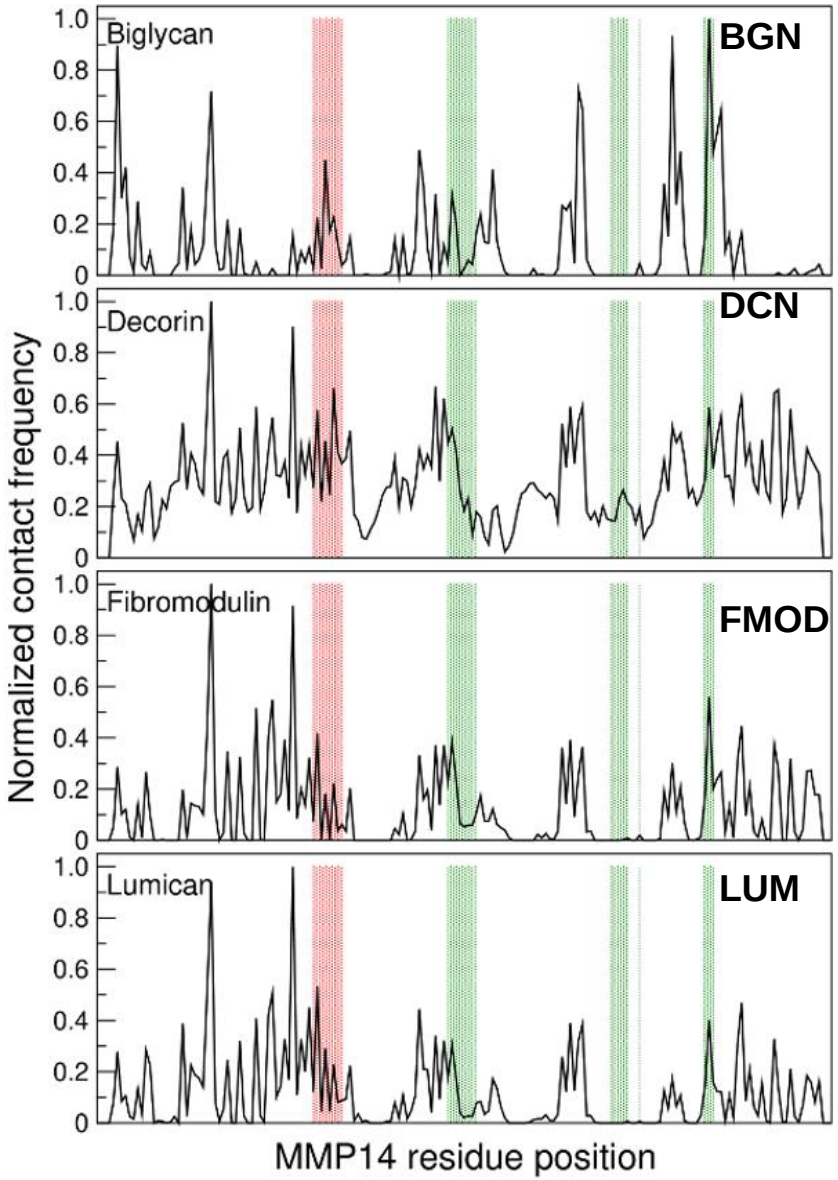


B

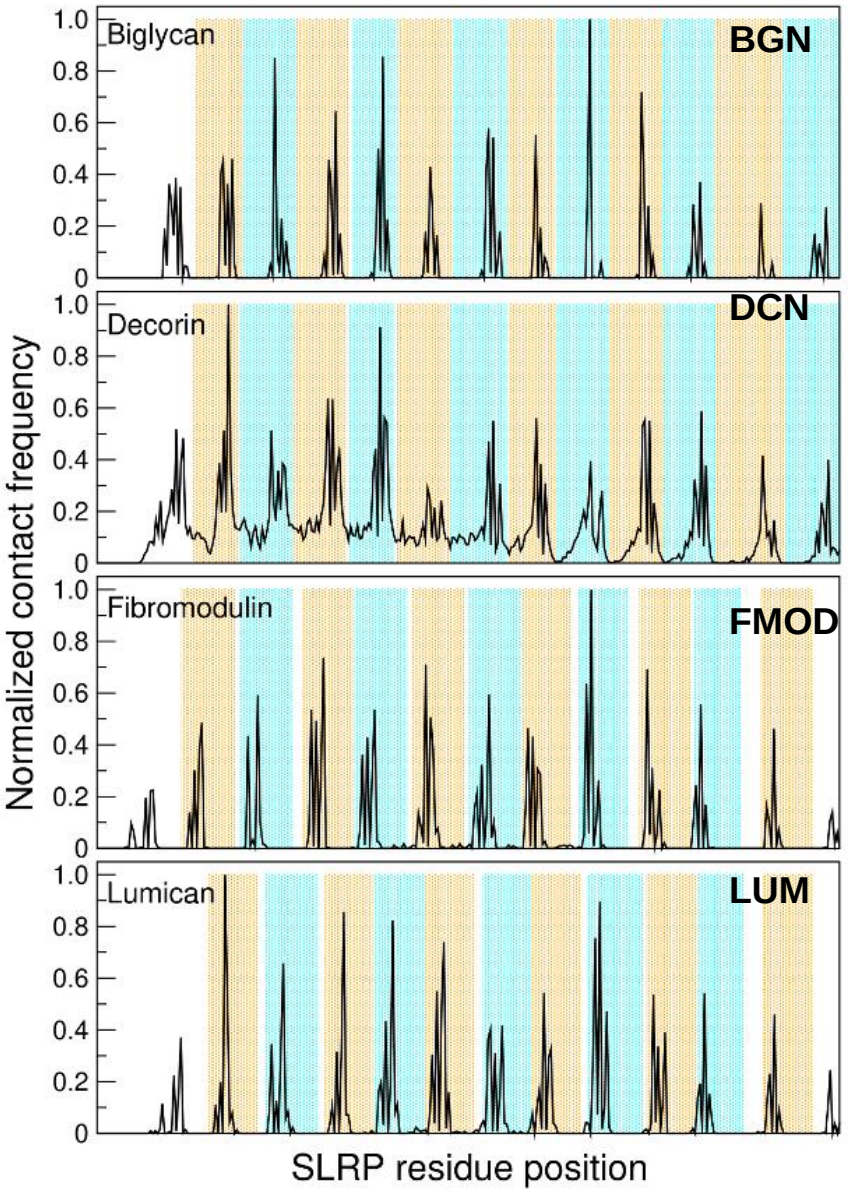


A**) MMP-14 domain structure** and **surface representation of the catalytic domain:**
The coordinates extracted from the pdb structure 1BQQ present a **catalytic pocket** and the **MT-LOOP**. The ASN229 is highlighted in orange as a possible N-glycosylation site.
Adapted from Pietraszek-Gremplewicz *et al.*, Matrix Biology, 2019

B) **MMP-14 catalytic domain complexes** formed with **human SLRPs (**BGN, DCN, FMOD, LUM**):**
The results were obtained with the HEX software and correspond to the best binding energy. Residues bearing the **N-glycosylations** are displayed using purple surfaces.
Rivet *et al*., submitted to AJP Cell Phys.



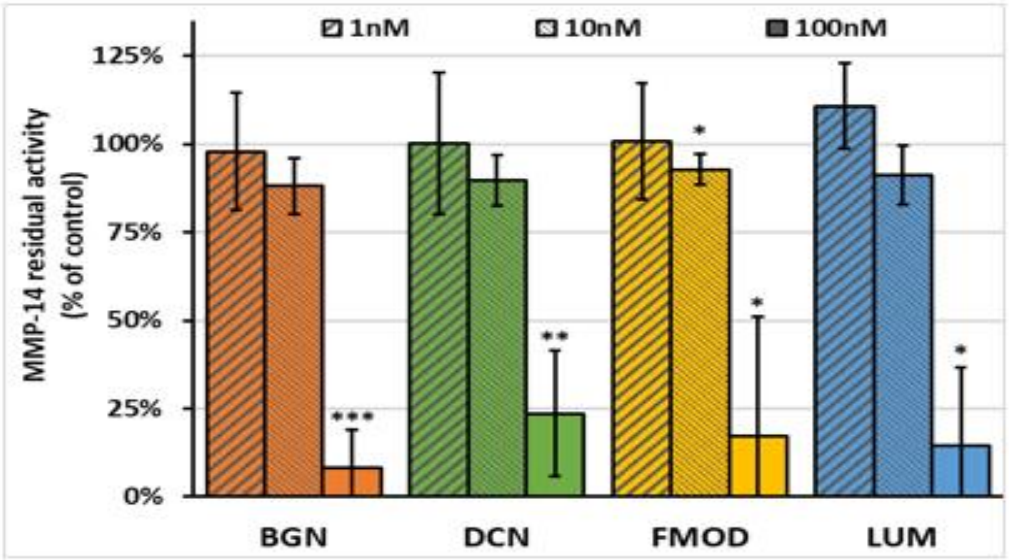
C) **MMP-14 residue** positions interacting with **SLRPs** in the **MT-LOOP** and in the **catalytic pocket** of **MMP-14.**



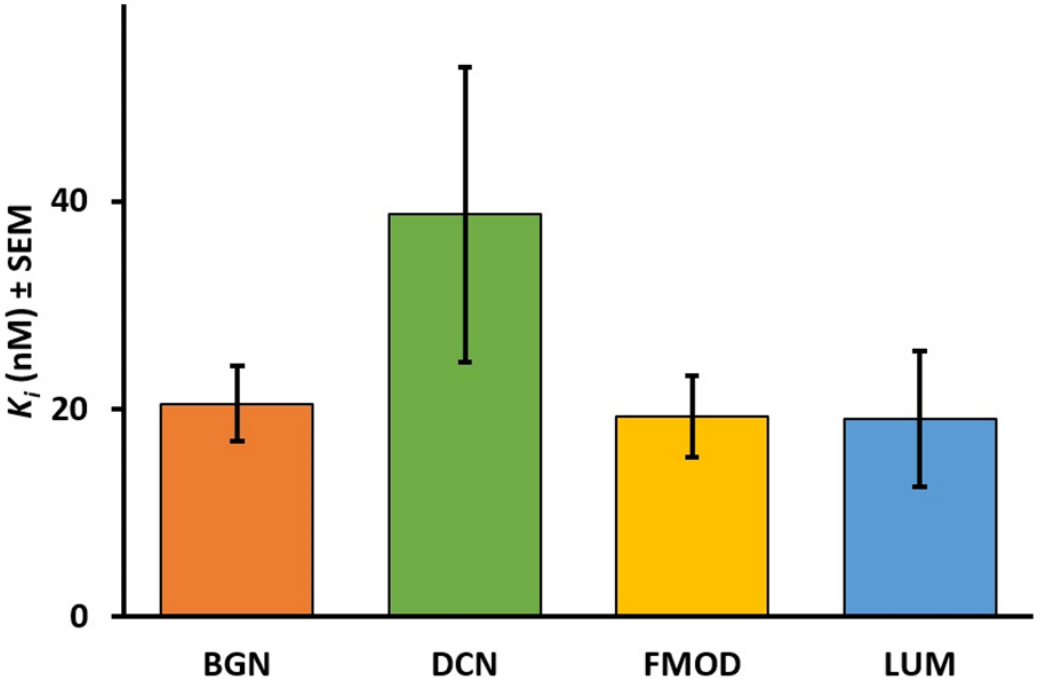
D) **SLRPs residue** positions interacting with **MMP-14.** The **LRRs** in **BGN, DCN, FMOD** and **LUM** are indicated in orange and blue, alternatively.

Rivet *et al*., submitted to AJP Cell Phys.

A) MMP-14 activity



B) K_i calculation



MMP-14 activity assay and measurement of K_i.

A) Effect of recombinant biglycan (BGN), decorin (DCN), fibromodulin (FMOD), and lumican (LUM) on recombinant MMP-14 activity measured with increasing concentrations of SLRPs (1, 10, 100 nM). Data are presented as mean values ± SD from four independent experiments.

B) Measurement of the K_i for each SLRP.

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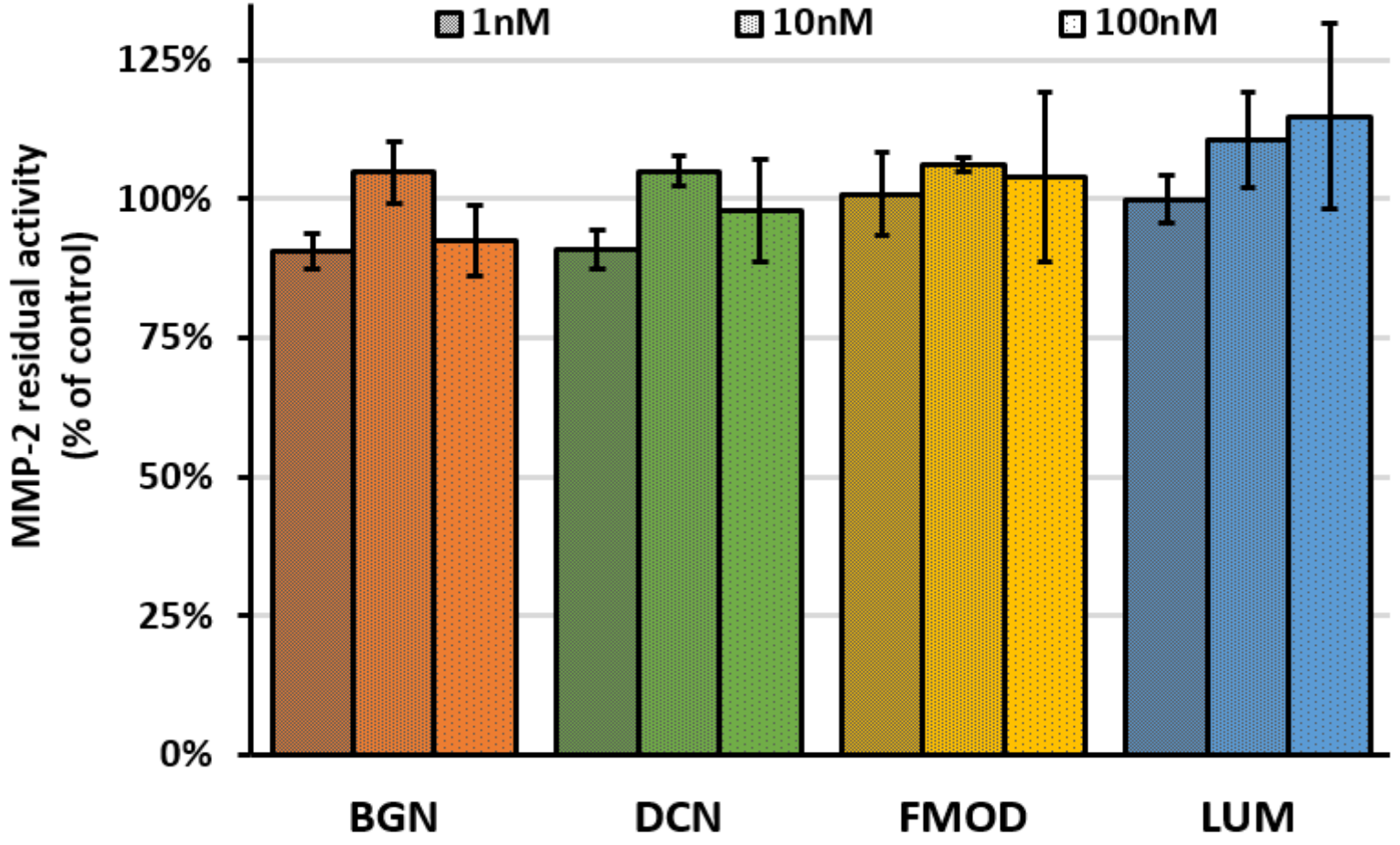
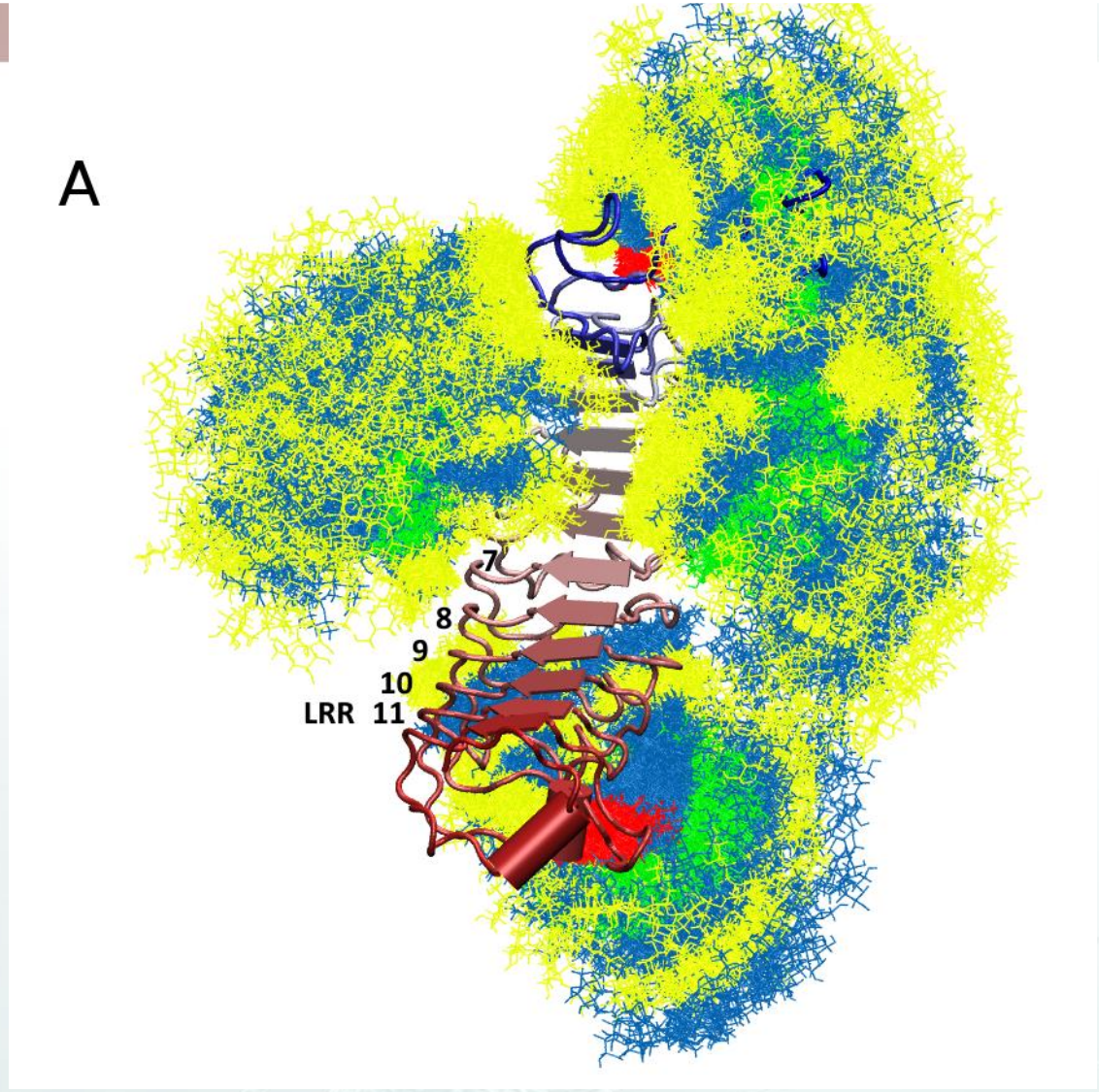
MMP-2 activity



MMP-2 activity assay.
Effect of recombinant biglycan (BGN), decorin (DCN), fibromodulin (FMOD), and lumican (LUM) on recombinant MMP-2 activity measured with increasing concentrations of SLRPs (1, 10, 100 nM).
Data are presented as mean values ± SD from three independent experiments.

Rivet *et al.*, submitted to AJP Cell Phys.

1) Impact of the carbohydrate shielding of lumican on LRR accessibility



D

		Glycosylated lumican			Core protein		
LRR	Residue	AccAr from MD (Å²)	RelAcc (%)	Accessibility	AccAr from starting structure (Å²)	RelAcc (%)	Accessibility
7	T209	11.9 ± 5.1	8.2	Buried	3.3	2.2	Buried
	L210	0.3 ± 1.0	0.0	Buried	0.0	0.0	Buried
	Y211	35.3 ± 8.5	15.4	Buried	31.3	13.6	Buried
9	E258	47.4 ± 11.1	25.9	Accessible	63.1	34.4	Accessible
	L259	0.1 ± 0.6	0.0	Buried	0.0	0.0	Buried
	D260	17.2 ± 4.8	11.2	Buried	22.1	14.5	Buried
11	H308	41.4 ± 15.1	21.1	Accessible	39.4	20.1	Accessible
	L309	0.5 ± 1.4	0.0	Buried	0.0	0.0	Buried
	R310	75.0 ± 12.0	31.1	Accessible	92.1	38.1	Accessible

D) Solvent accessible surface areas (AccAr) and relative accessibility (RelAcc) of selected lumican residues that are part of LRR7, LRR9 and LRR11.

A residue is considered buried when its RelAcc is < 20 and it is considered accessible when its RelAcc is > 20.

>> improved accessibilities upon glycosylation

A) Front view showing the solvent-unaccessible as well as solvent accessible LRRs.
The C-terminal LRRs (LRR-7 to LRR-11) are labelled adjacent to the respective β-strands.

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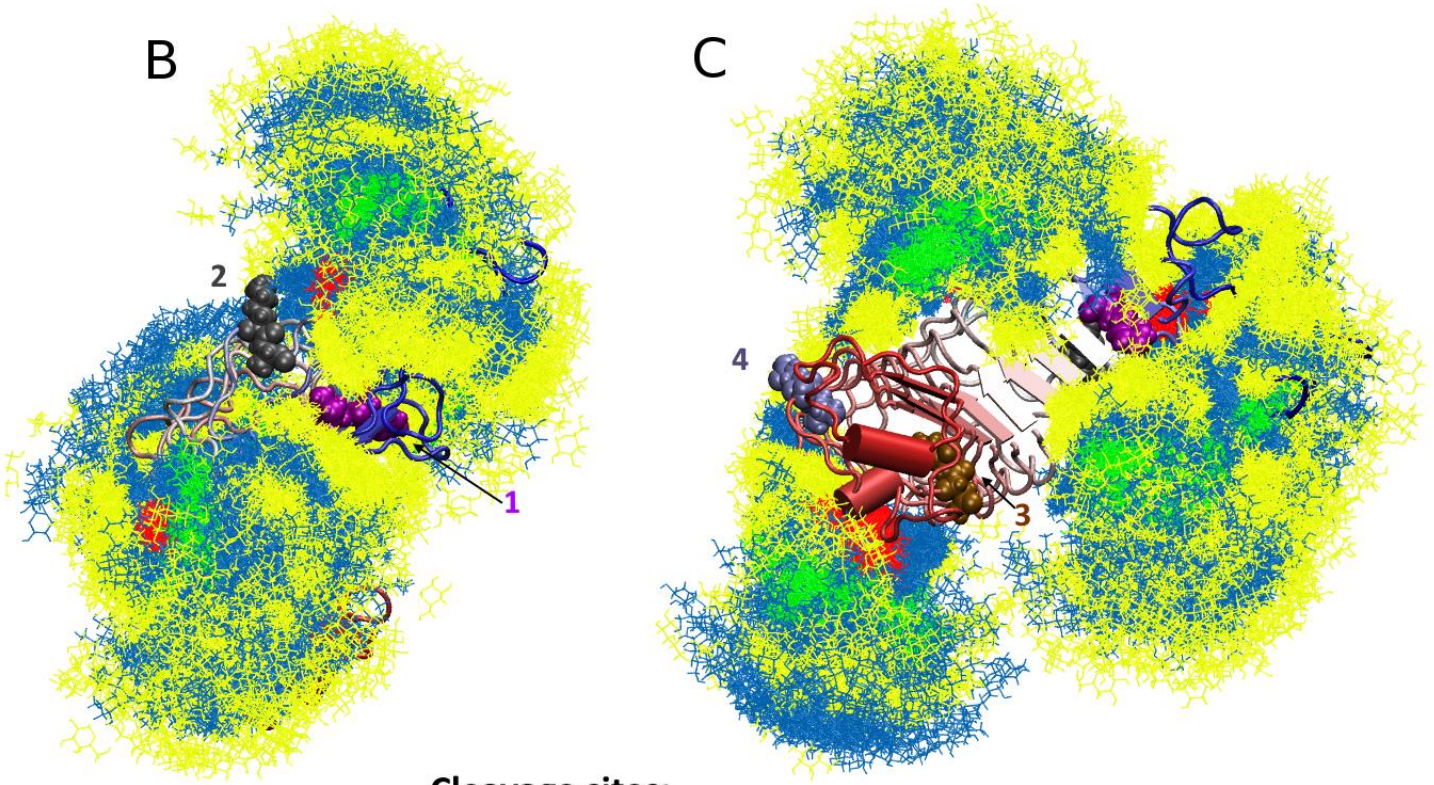
2) Impact of the carbohydrate shielding of lumican on MMP-14 cleavage sites accessibility



Cleavage sites:

1: 70-YL-71

2: 84-KA-85

3: 275-NL-276

4: 285-QL-286

E

		Glycosylated lumican			Core protein		
Cleavage site	Residue	AccAr from MD (Å²)	RelAcc (%)	Accessibility	AccAr from starting structure (Å²)	RelAcc (%)	Accessibility
1 (in LRR 1)	Y70	38.7 ± 18.7	16.8	Buried	53.8	23.4	Accessible
	L71	0.4 ± 1.3	0.0	Buried	0.0	0.0	Buried
2 (in LRR 1)	K84	160.5 ± 2.4	75.0	Accessible	141.3	66.0	Accessible
	A85	14.9 ± 13.5	13.1	Buried	23.3	20.6	Accessible
3 (in LRR 9)	N275	65.4 ± 12.9	41.3	Accessible	84.9	53.7	Accessible
	L276	0.5 ± 1.2	0.0	Buried	0.0	0.0	Buried
4 (in LRR 10)	O285	80.0 ± 18.3	42.3	Accessible	71.9	45.5	Accessible
	L286	0.3 ± 1.0	0.0	Buried	0.0	0.0	Buried

E) Solvent accessible surface areas (AccAr) and relative accessibility (RelAcc) of lumican residues situated in the cleavage sites. A residue is considered buried when its RelAcc is < 20, and it is considered accessible when its RelAcc is > 20.

>> decreased accessibilities upon glycosylation

B) Top-view showing the N-terminal half of lumican with the residues of cleavage sites 1 and 2 represented as Van der Waals (VdW) spheres.

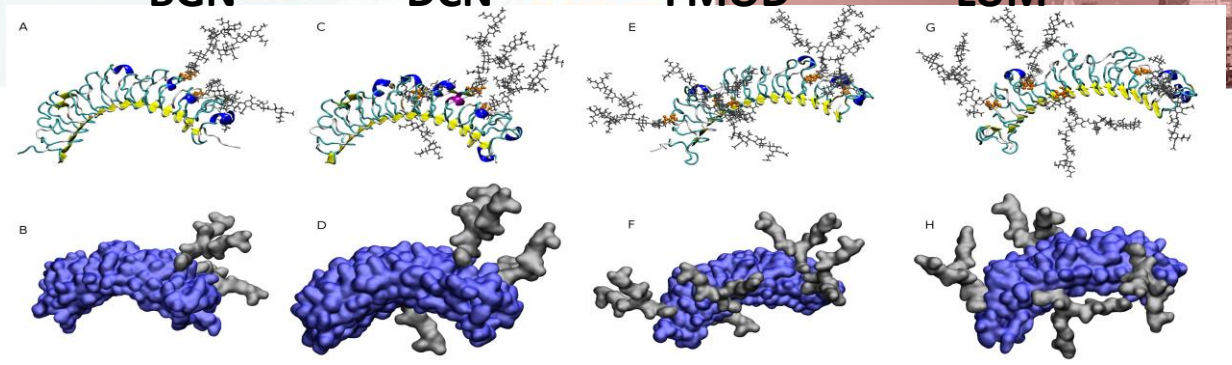
C) Bottom-view showing the C-terminal half of lumican with the residues of cleavage sites 3 and 4 represented as VdW spheres.

Protein is represented as cartoon, coloured in blue-white-red scheme (N-terminal to C-terminal), and carbohydrate residues are represented as sticks and coloured according to the SNFG scheme [Varki, Proteomics 2009]. The cleavage sites were taken from the experimental studies on lumican proteolysis by MMP-14 [Li, Cancer Research 2004].

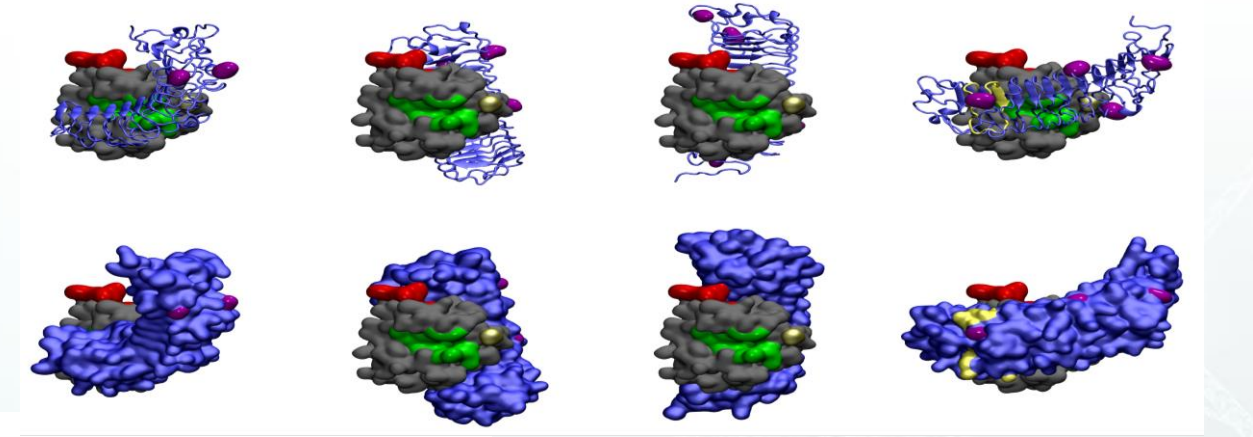
Rivet *et al*., submitted to AJP Cell Phys.

Key findings:

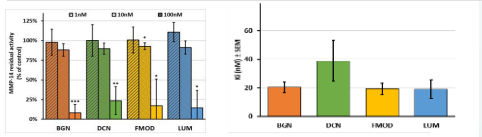
- **Secondary structures and N-glycosylation positions on human biglycan, decorin, fibromodulin and lumican:**



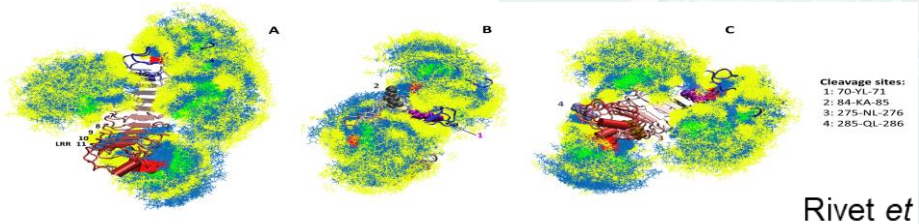
- **Rigid SLRPs/MMP-14 docking Experiments:**



- **MMP-14 activity assay and measurement of K_i:**



- **Carbohydrate shielding of Lumican:**



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ACKNOWLEDGEMENTS

MEDyc: Extracellular Matrix and Cell Dynamic, CNRS/URCA UMR N°7369; Head: Pr. L. Martiny

Team 1

Extracellular matrix, Cancer and therapeutic targets **(S. Dedieu, S Brézillon)**

Proteoglycan group:
S. Brézillon, L. Ramont, R. Rivet, P. Nizet,
C. Colin-Pierre , C. Sellier, I. Proult, L. Huber

Team 2

- Matrix aging and vascular remodeling (L. Duca, S. Jaisson)

Team 3

- **Modeling and multiscale imaging (S. Baud, M. Dauchez)**

S. Baud, M. Dauchez, N. Belloy, R. M. Rao, L. Debelle, J-M.Crowet, J. Prevoteau-Jonque

P3M MAISON DE LA SIMULATION de Champagne-Ardenne

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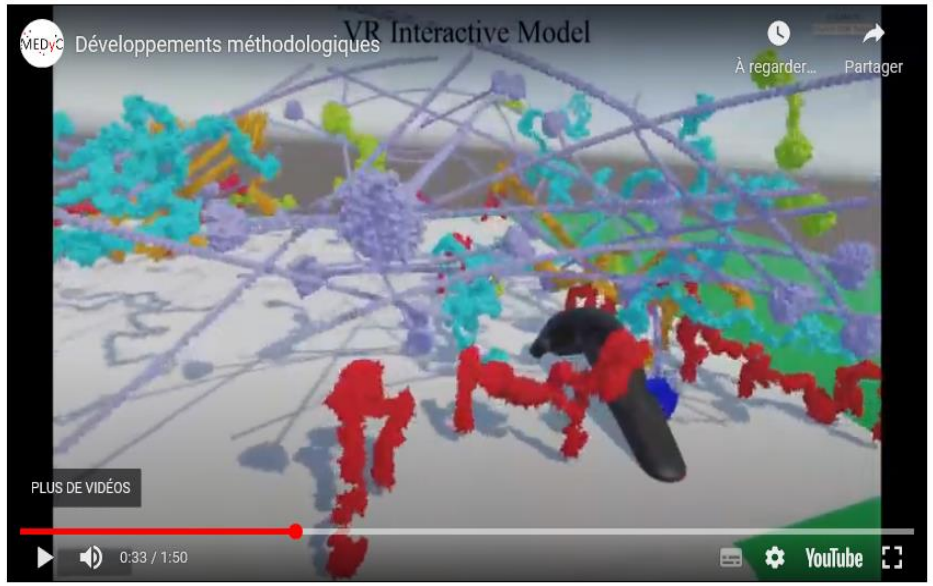
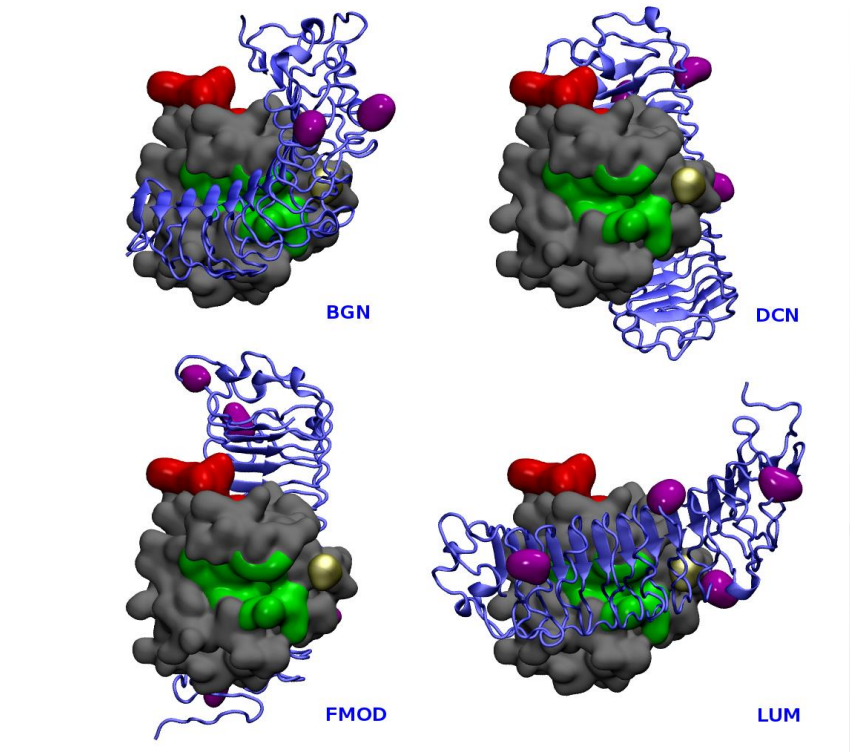


Thanks for your attention !

See posters: N°99 N°118

Differential MMP-14 targeting by Biglycan (BGN), Decorin (DCN), Fibromodulin (FMOD), and Lumican (LUM) unraveled by *In Silico* Approach

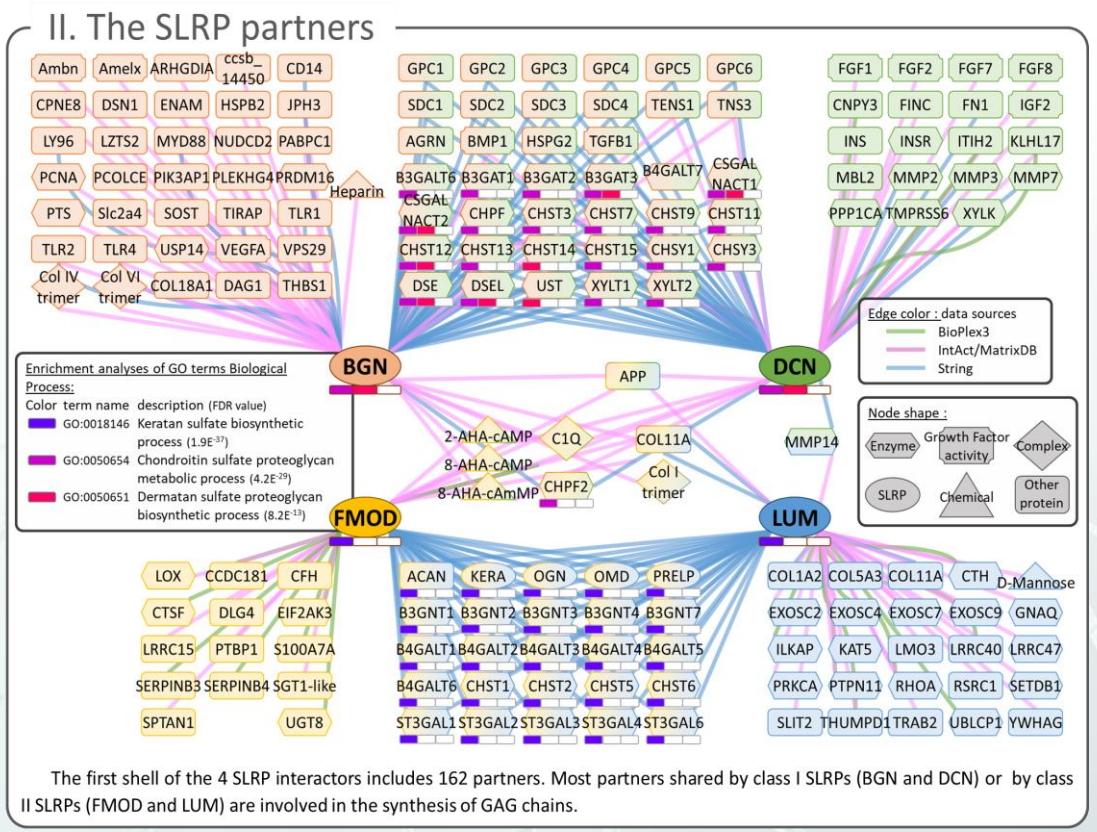
R. Rivet, R. M. Rao, P. Nizet, N. Belloy, L. Huber, M. Dauchez, L. Ramont, S. Baud, S. Brézillon



Rivet *et al*., submitted to AJP Cell Phys.

The interaction network of Biglycan, Decorin, Fibromodulin and Lumican

R. Rivet, S. Ricard-Blum, L. Ramont, S. Brézillon



Rivet *et al*., in preparation for FEBS J