Poster n°047

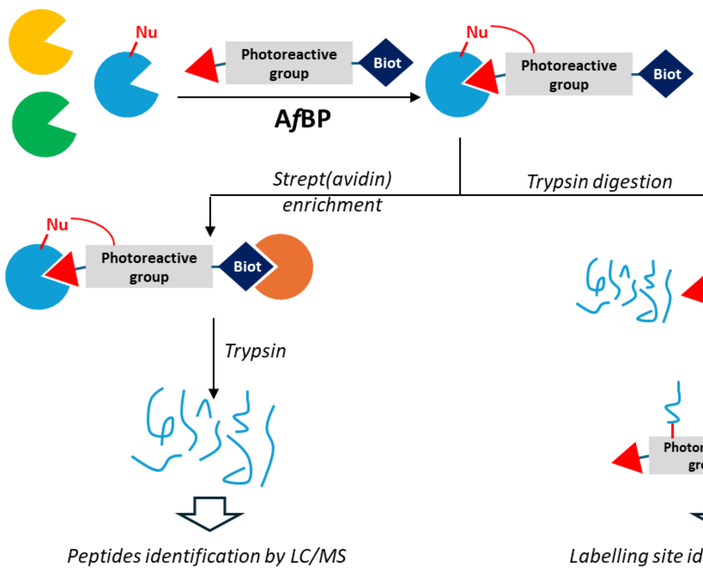
Affinity-driven transfer of a mass tag for the direct profiling of matrix metalloproteases in complex biological matrices

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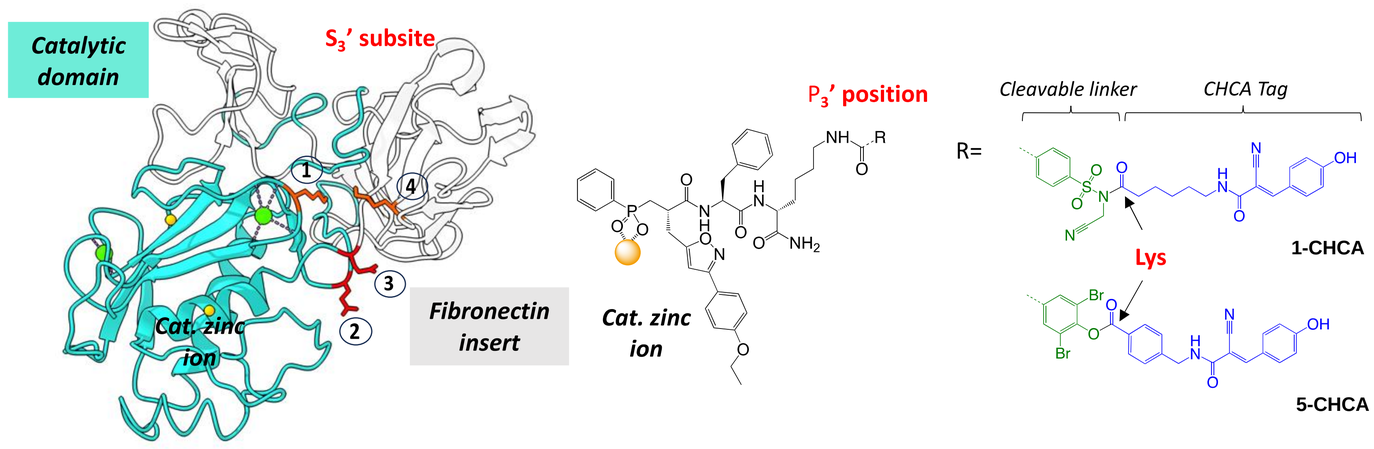
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Among the diverse family of proteases, matrix metalloproteinases (MMPs) are a notable subset of 23 zinc-dependent endopeptidases. MMPs play essential roles in numerous physiological and pathological processes[1]. These enzymes are initially synthesized as inactive zymogens and require activation in the pericellular or extracellular environment. Activated MMPs process a broad spectrum of substrates, including components of the extracellular matrix (ECM) and non-ECM molecules, influencing tissue architecture and cellular functions. Under normal physiological conditions, MMP activity is tightly regulated by endogenous inhibitors; however, this balance is often disrupted in disease states. MMPs also initiate complex proteolytic cascades, activating other MMPs and generating novel substrates[2]. Detecting the activation profile of MMPs in specific pathological contexts is crucial for elucidating their precise functions and confirming their potential pathological roles, which remain unclear in many diseases where these proteases are overexpressed.

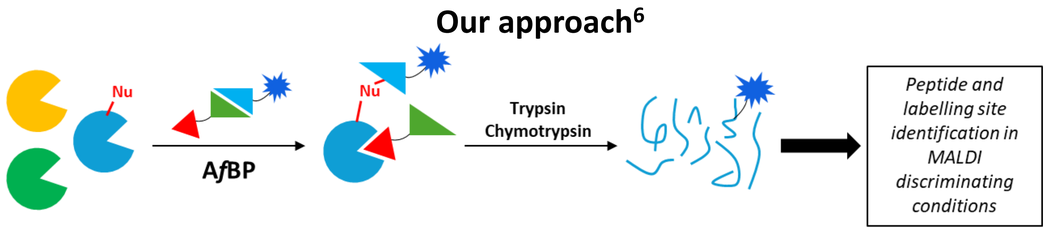
Traditional approaches for the profiling of metalloproteases



Structural context and design of the probes



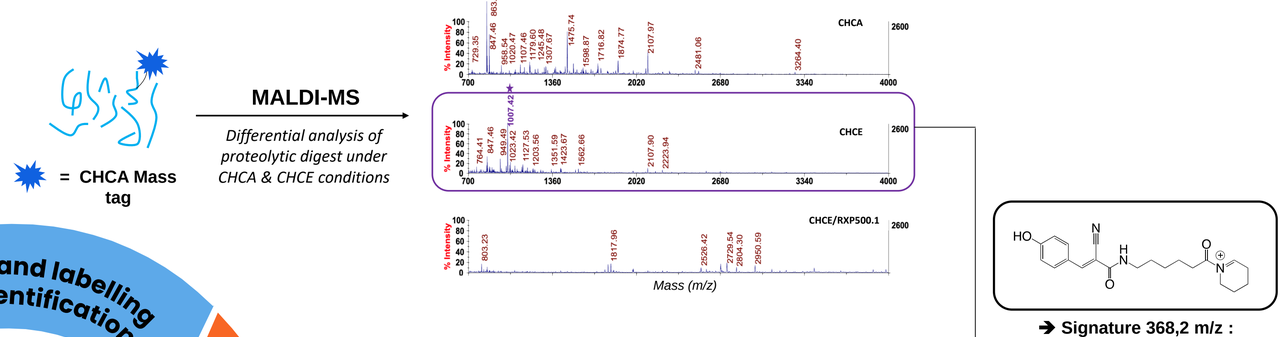
Our approach[6]

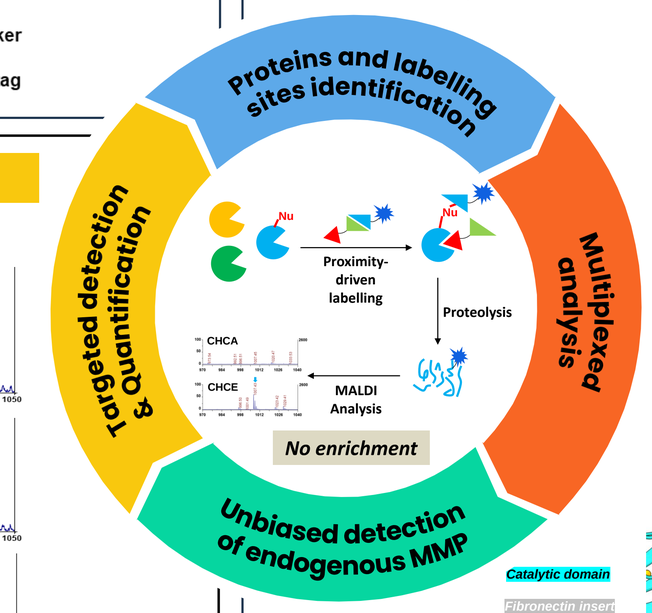


- ✓ No enrichment step required
- ✓ Qualitative & Quantitative profiling
- ✓ Photoactivation-free process of labelling[3, 4, 5]

= Recognition motif
= Cleavable linker
= CHCA Mass tag

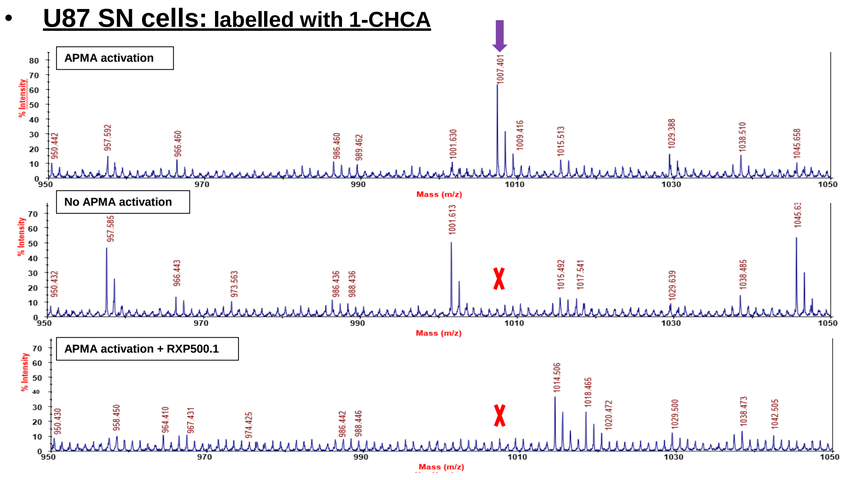
Labelled MMPs are associated to characteristic signature peptides





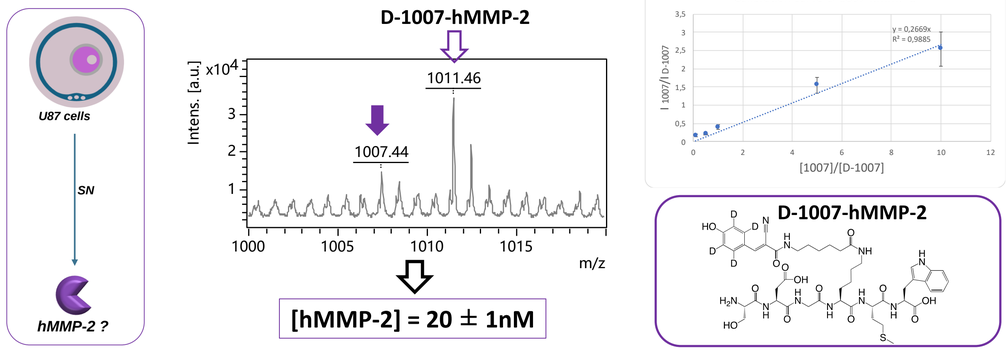
hMMP2 in U87 cells supernatant

- **U87 SN cells: labelled with 1-CHCA**



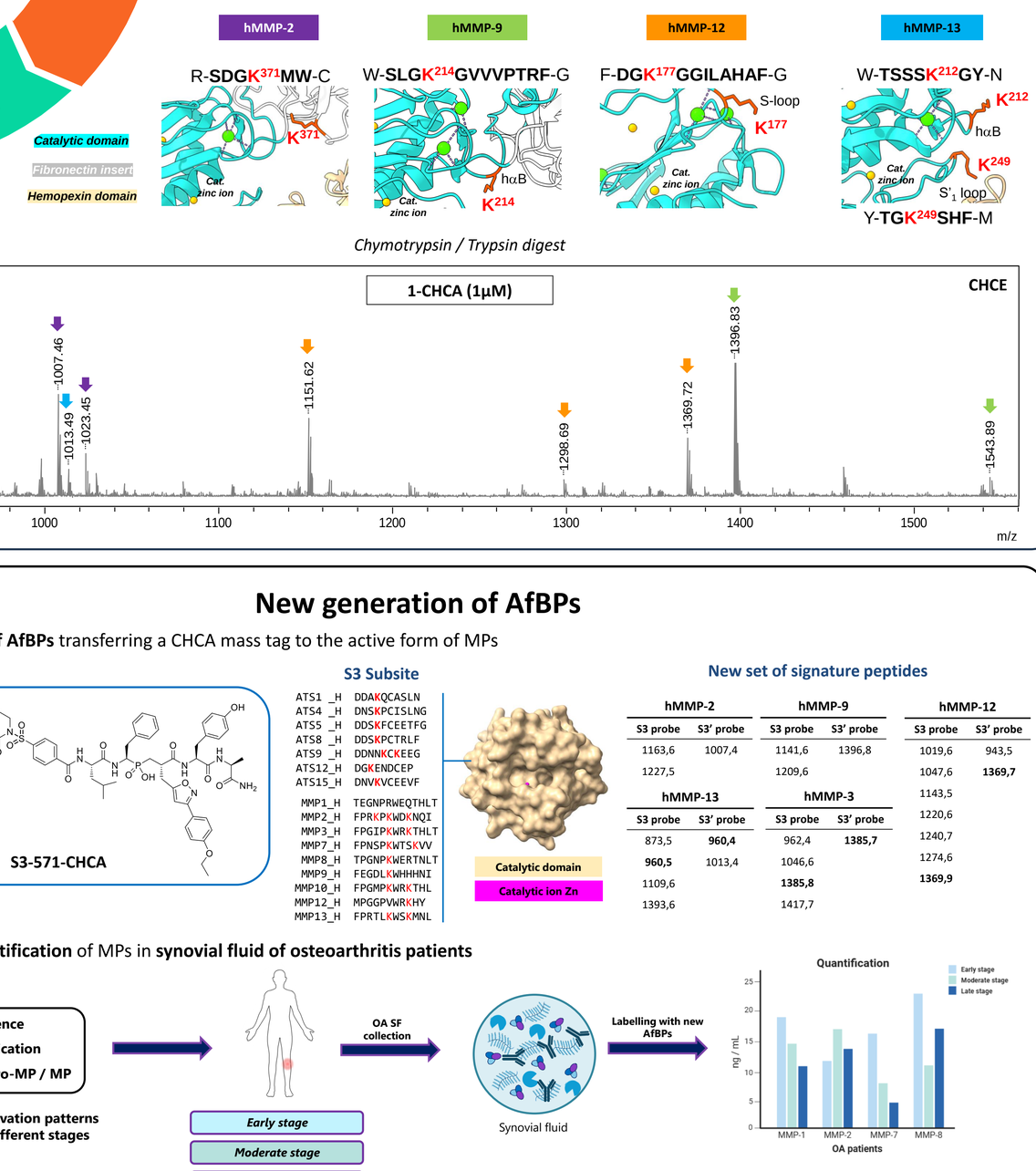
➔ hMMP-2 detected in U87 SN after APMA activation

- **Quantitative profiling**



hMMP3 in chondrocytes supernatant

Multiplexed detection of MMP-2, -9, -12 & -13



New generation of AfBPs

- **New generation of AfBPs** transferring a CHCA mass tag to the active form of MPs

S3 Subsite

ATS1 _H	DDAKQCASLN
ATS4 _H	DNSKPCISLNG
ATS5 _H	DDSKFCEETFG
ATS8 _H	DDSKPCTRLF
ATS9 _H	DDNNKCKEEG
ATS12_H	DGKENDCEP
ATS15_H	DNVKVCEEVF
MMP1_H	TEGNPRWEQTHLT
MMP2_H	FPRKPKWDKNQI
MMP3_H	FPGIPKWRKTHLT
MMP7_H	FPNSPKWTSKVV
MMP8_H	TPGNPKWERTNLT
MMP9_H	FEGDLKWHHHNI
MMP10_H	FPGMPKWRKTHL
MMP12_H	MPGGPVWRKHY
MMP13_H	FPRTLKWSKMNL

New set of signature peptides

hMMP-2		hMMP-9		hMMP-12	
S3 probe	S3' probe	S3 probe	S3' probe	S3 probe	S3' probe
1163,6	1007,4	1141,6	1396,8	1019,6	943,5
1227,5		1209,6		1047,6	**1369,7**
hMMP-13		**hMMP-3**		1143,5	
S3 probe	S3' probe	S3 probe	S3' probe	1220,6	
873,5	**960,4**	962,4	**1385,7**	1240,7	
960,5	1013,4	1046,6		1274,6	
1109,6		**1385,8**		**1369,9**	
1393,6		1417,7			

- **Detection** & **Quantification** of MPs in **synovial fluid of osteoarthritis patients**

Conclusion & Perspectives

- **New generation of AfBPs** transferring a CHCA mass tag to the active form of MMPs.
- Identification of CHCA-labelled MMPs & determination of their labelling sites within **a single workflow**.
- New ABPP to characterize the complete **activation pattern of MMPs** in a specific pathological context.
- This approach could be expanded for **probe-based target deconvolution** within **a single workflow**.
- Significant potential for the **rapid analysis of multiple proteins** in complex proteomes.
- By leveraging the MALDI-TOF format, our approach could be applied to multiple pathological fluids, ultimately serving as a **robust diagnostic platform.**

[1]De Almeida, L. G. N. *et al. Pharmacological Reviews* **74**, 714–720 (2022). [2]Kollet, O., Das, A., Karamanos, N., Auf Dem Keller, U. & Sagi, I. *Trends in Molecular Medicine* **30**, 147–163 (2024). [3]Kaminska, M. *et al. Angew Chem Int Ed* **60**, 18272–18279 (2021). [4]Malgorn, C. *et al. Proteases and Cancer* (ed. Santamaria, S.) vol. 2747 29–39 (Springer US, New York, NY, 2024). [5]Devel, L. *et al. ChemBioChem* **25**, e202400441 (2024). [6]Berthy, L., Pasco U. *et al. Angew Chem Int Ed* **65**, e23132 (2026).