Engineering the MALDI interface for in situ detection of modified peptides in synovial fluids : application to osteoarthritis diagnosis

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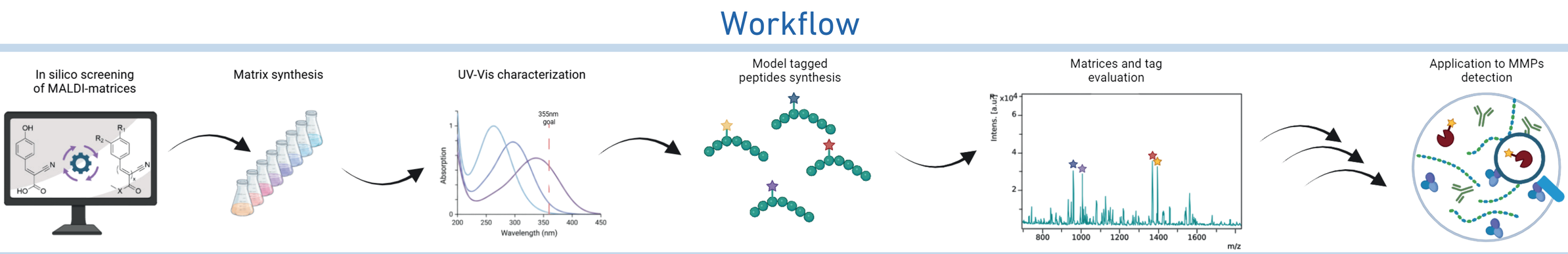
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Introduction

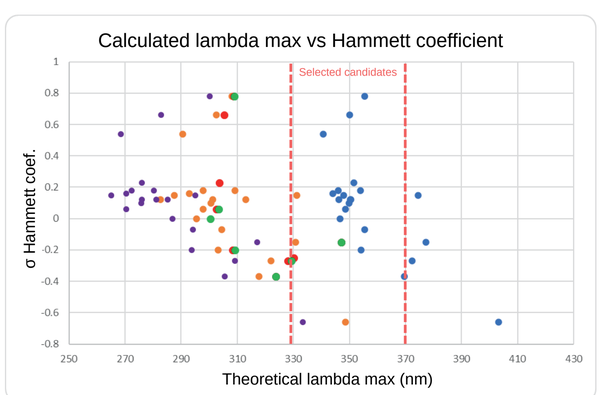
Osteoarthritis is one of the most common joint diseases, but its **treatment remains limited**, largely due to late diagnosis. It is usually detected only when pain appears, by which point significant tissue damage has already occurred.[1] This degradation is attributed to increased concentration of **active matrix metalloproteinases** (MMPs), making them promising **early biomarkers**, but their detection remains challenging because of their low abundance and the coexistence of inactive forms and active forms.[2,3]

Here, we present a sensitive and selective **MALDI-MS based approach** to detect active MMPs in synovial fluid. The method relies rational design of **aprotic MALDI matrices** combined with specific **mass-tag probes**, allowing selective **detection of active enzymes down to the nanomolar range**.[4] This approach highlights the potential of tailored MALDI-MS strategies for early OA biomarker detection.

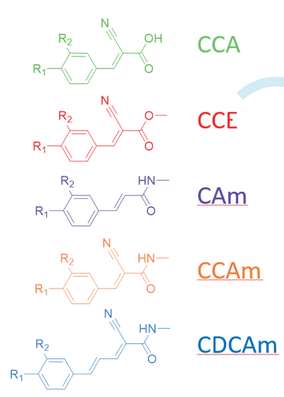
Workflow



Screening of five classes of molecules (n=79)

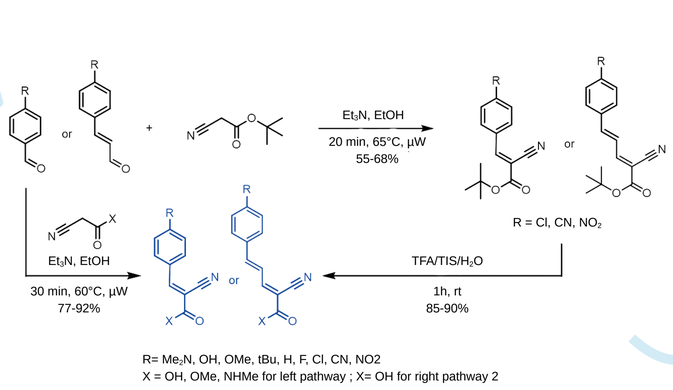


Graph showing predicted lambda max of 79 molécules over Hammett coef.[5] DFT used for geometry optimization and TD-DFT for excited states energies and lambda max calculation using B3LYP/def2-SVG as functional and basis



R1 = NH_2, OH, OMe, tBu, CH_3, H, F, $SiCH_3$, CH_2Cl, SH, CH_2CN, I, Cl, CF_3, CN, NO_2
R2 = OMe, OH, SCH_3, CH_2CN

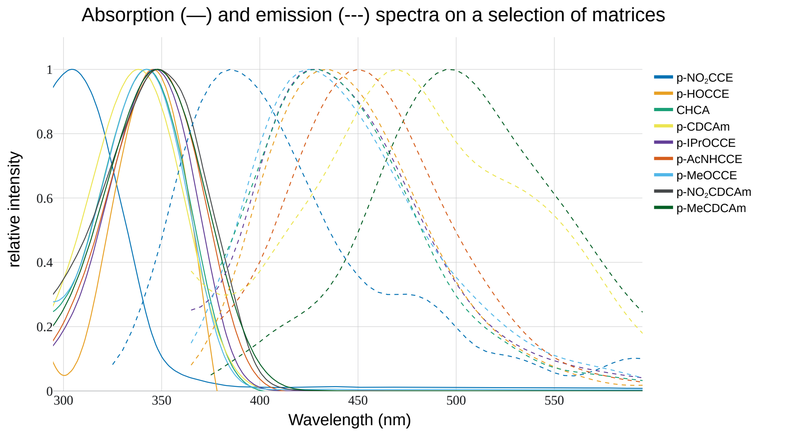
Synthesis of selected candidates (n=30)



R= Me_2N, OH, OMe, tBu, H, F, Cl, CN, NO2
X = OH, OMe, NHMe for left pathway ; X= OH for right pathway 2

Synthetic pathways of selected matrices and tags. For acidic matrices and tags with strong para-EWG, protection was necessary to prevent decarboxylation.

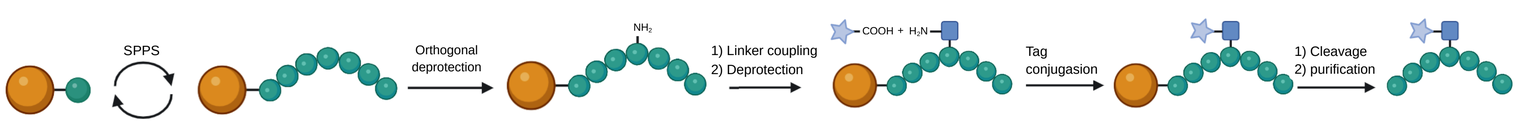
UV-Visible characterization (n=16)



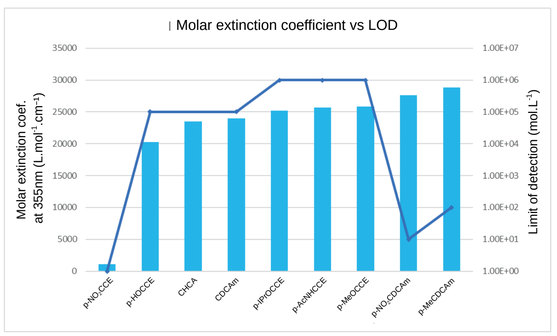
Emission spectra were recorded from 200 to 800 nm and normalized to the maximum absorption of each compound. The emission scans were collected from λmax +30 nm to 800 nm, using an excitation wavelength set to each compound's maximum absorption wavelength.

Matrices and tags evaluation (n=9)

Model peptides synthesis



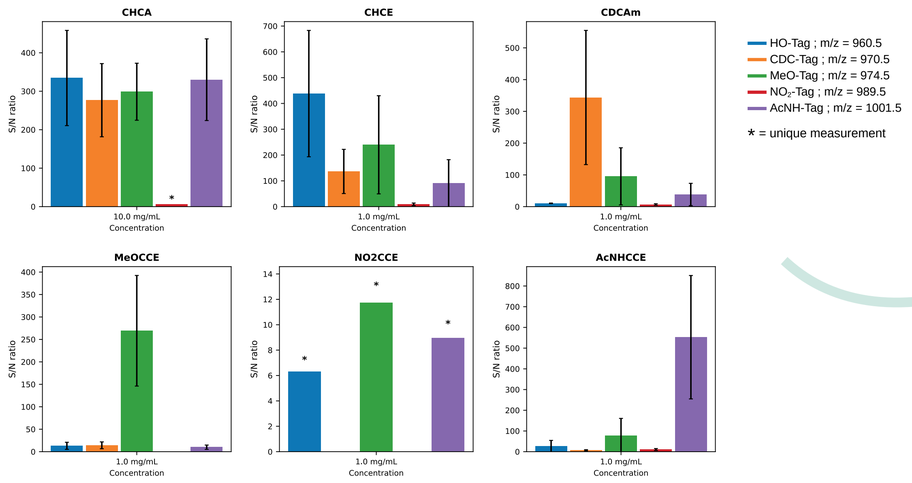
LODs measurement



Molar extinction coefficients were measured at 355 nm from 5-point calibration curves (n=3 per point). Limits of detection (LOD) were determined by serial dilution of the pure peptide (10^{-7}-10^{-14} M) analyzed on CHCA (10 mg/mL) and the corresponding para-substituted matrix (1 mg/mL). Results are expressed as fold-increase in sensitivity relative to CHCA

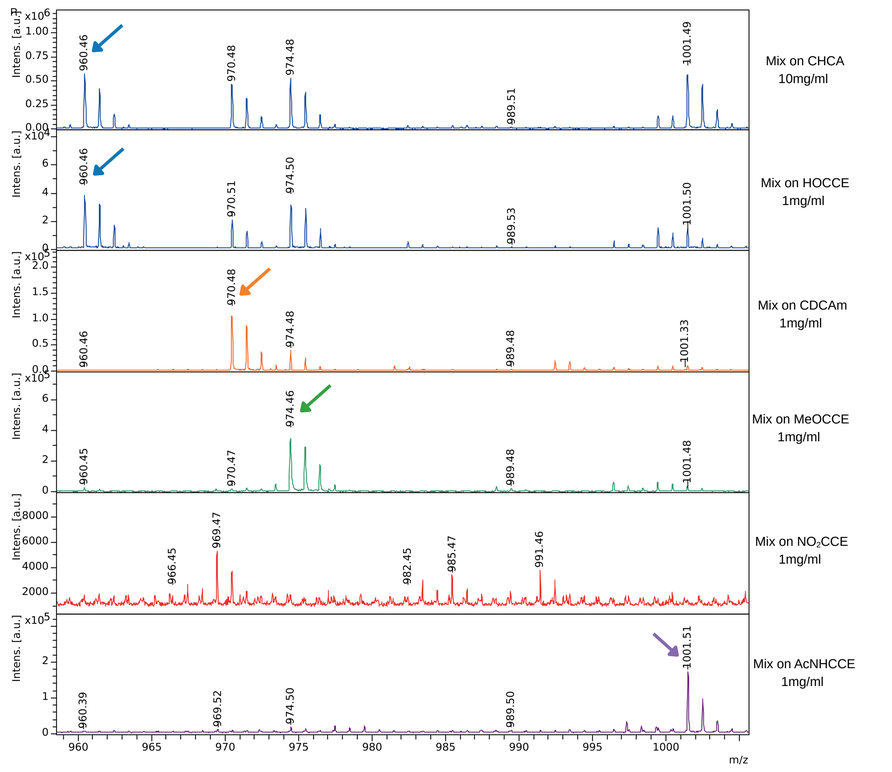
A higher molar extinction coefficient initially results in a higher limit of detection. However, above 27000 L·mol⁻¹·cm⁻¹, the limit of detection decreases, potentially due to matrix effects and/or photodegradation of the mass tag.

Ionisation selectivity



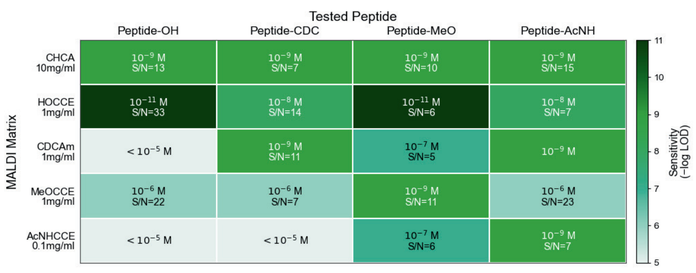
S/N ratios for each tagged peptide were extracted from MALDI spectra. An equimolar peptide mix at 10^{-7}M was analyzed on 6 different matrices : CHCA 10mg/ml and engineered ones at 1 mg/mL, using the same substitutions as the tags (3 replicates per condition). Bars represent individual tags; boxes represent matrices.

MALDI-MS spectra

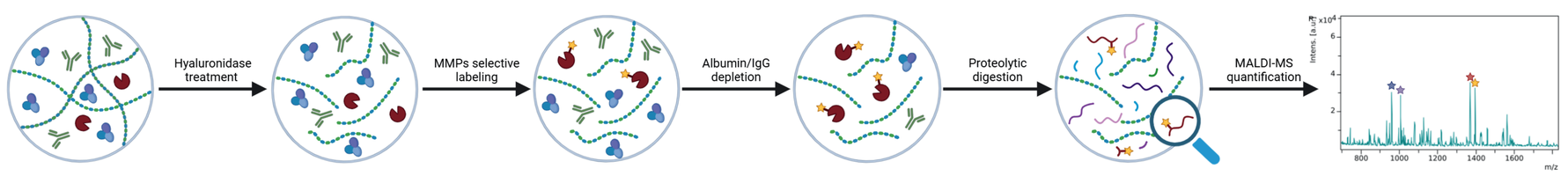


Application to MMPs detection in OA (n=4)

Heatmap of peptide's LODs in synovial fluid



Treatment of synovial fluid for MALDI-MS analysis



Synthetic synovial fluid was purchased to optimize our treatment pipeline. Viscosity was first reduced from 0.1 to 0.01 Pa·s using hyaluronidase (1 µg/ml). Tailored affinity-based probes[5] (AfBPs) were then used for selective labeling of hMMPs (37°C, 1 µM, 4h), followed by immunodepletion of albumin and gamma globulins to simplify the medium (from 0.7 g/l to 10 µg/ml). Samples were then digested with trypsin (1:16 enzyme/protein ratio, 2h, 50°C) followed by chymotrypsin (1:32, 2h, 50°C). Samples are then desalted on zip-tip column and analysed in MALDI mass spectrometry.

Take home messages

Matrix design

Among 79 screened structures, 30 were synthesized and 9 selected for MALDI-MS evaluation. Lamdba max were predicted with a mean error of 10nm.

Matrix and tag interplay

High molar extinction coefficients did not systematically translate into improved MALDI performance, with optimal performance observed around 25,000 L·mol⁻¹·cm⁻¹.

Biological application

A synovial-fluid treatment pipeline was established, enabling LOD determination of peptides bearing the four best-performing tags.

Perspectives

Validation in human synovial fluid samples and extension of the approach to active MMP profiling for early osteoarthritis detection.

References

[1] Mobasheri, A. et al. Biomarkers for osteoarthritis: Current status and future prospects. Best Practice & Research Clinical Rheumatology 37, 101852 (2023). **[2]** Stamenkovic, I. Extracellular matrix remodelling: the role of matrix metalloproteinases. The Journal of Pathology 200, 448–464 (2003). **[3]** Almeida, L. G. N. de et al. Matrix Metalloproteinases: From Molecular Mechanisms to Physiology, Pathophysiology, and Pharmacology. Pharmacological Reviews 74, 714–770 (2022). **[4]** Berthy, L. et al. Proximity-Induced Transfer of a Mass Tag Enables Direct Profiling of Active Matrix Metalloproteases. Angewandte Chemie International Edition 65, e23132 (2026). **[5]** Hansch, Corwin., Leo, A. & Taft, R. W. A survey of Hammett substituent constants and resonance and field parameters. Chem. Rev. 91, 165–195 (2002).