Spatio-temporal control of cellular differentiation for cartilage tissue engineering

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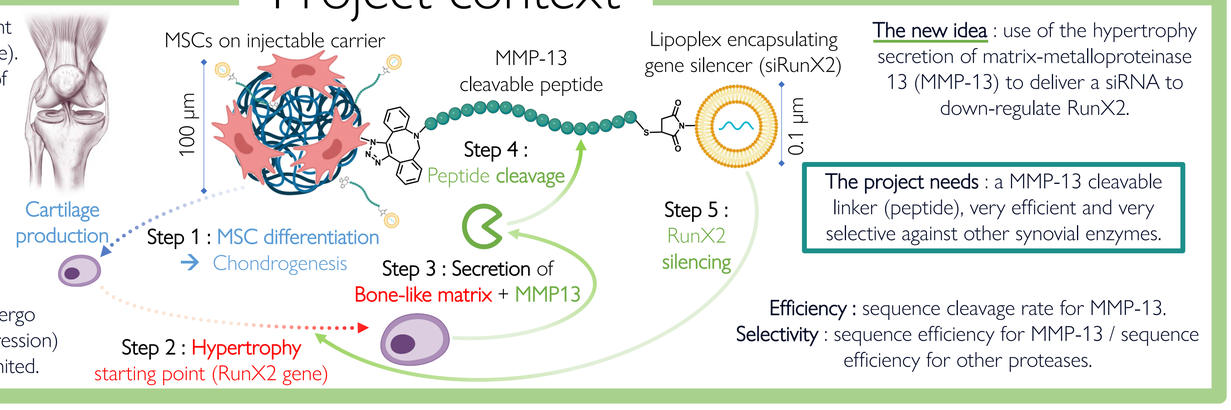
Project context

Osteoarthritis (OA) : most prevalent joint disease (1/13 people worldwide). Defined by self-healing difficulties of cartilaginous lesions.

Our approach to treat OA : deliver cartilage producing cells (chondrocytes) to the chondral lesions.

The problem : chondrocytes undergo hypertrophy (RunX2 gene overexpression) and production of collagen II is limited.



The new idea : use of the hypertrophy secretion of matrix-metalloproteinase 13 (MMP-13) to deliver a siRNA to down-regulate RunX2.

The project needs : a MMP-13 cleavable linker (peptide), very efficient and very selective against other synovial enzymes.

Efficiency : sequence cleavage rate for MMP-13.
Selectivity : sequence efficiency for MMP-13 / sequence efficiency for other proteases.

Poster summary

In this work, we present a new methodology for rapid screening of protease-cleavable peptide with high selectivity.

1. First, we designed a program capable of outputting protease-selective sequence libraries.
2. Then, we developed a fast and accurate method for the screening of efficiency and selectivity of peptide libraries.
3. Finally, we validated the program and the new screening method with established screening method (by UHPLC).

1 Computer Program and analysis optimizations

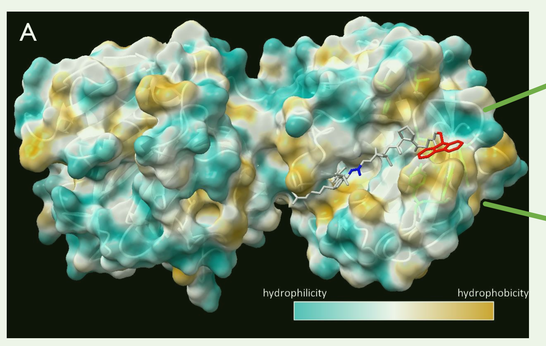
Programs available : **SELECTIVITY**
- SitePrediction and PeptideCutter (cleavage site)
- PepSite (binding sites)
- DeepPeptide (AI) and PoPS (substrate specificity)

Our program : **SELECTIVITY** **EFFICIENCY**

- based on the **score of amino acids**, per position and per enzyme (MEROPS), gives :
- **the most selective sequence** of the target protease
- **the sequence library** from the **most selective** to the **most efficient**
- **the most efficient protease** of the target sequence

n°	Sequences	n°	Sequences	n°	Sequences
1	GPKG**X**MNPP	9	GPPG**X**MNPP	17	GPLG**X**MNPP
2	GPKG**X**MNGP	10	GPPG**X**MNGP	18	GPLG**X**MNGP
3	GPKG**X**MRPP	11	GPPG**X**MRPP	19	GPLG**X**MRPP
4	GPKG**X**MRGP	12	GPPG**X**MRGP	20	GPLG**X**MRGP
5	GPKG**X**LNPP	13	GPPG**X**LNPP	21	GPLG**X**LNPP
6	GPKG**X**LNGP	14	GPPG**X**LNGP	22	GPLG**X**LNGP
7	GPKG**X**LRPP	15	GPPG**X**LRPP	23	GPLG**X**LRPP
8	GPKG**X**LRGP	16	GPPG**X**LRGP	24	GPLG**X**LRGP

24-peptide library
Target protease : MMP-13
Competing proteases : [MMP-1, MMP-2, MMP-3, MMP-9, ADAMTS-4, ADAMTS-5]
" **X** " : sequence cleavage bond



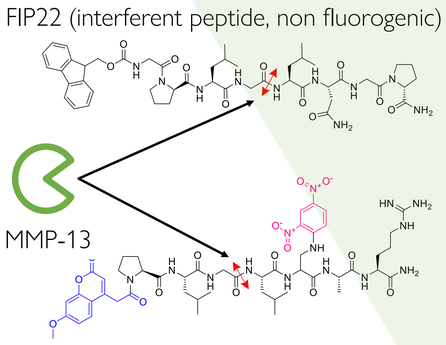
An aromatic area was found near the cleavage site, with matching distances (N-ter--cleavage bond and Zn--aromatic area). All further studies were made with Fmoc N-ter peptides (FIPs) to allow peptides to interact with MMP-13.

MMP-13 modelization
A : Enzyme with peptide in the catalytic pocket
B : Catalytic site zoom
— : 15 A
— : 14,5 A

2.a Indirect Fluorescence Competition (IFC) : Efficiency

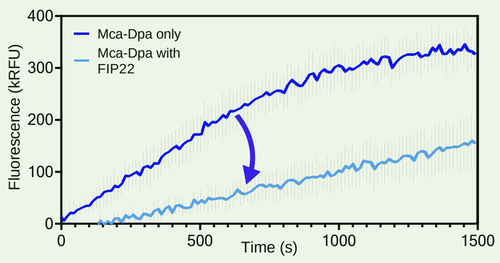
Principle

FIP22 (interferent peptide, non fluorogenic)



Mca-Dpa (MMP substrate, fluorogenic)

FIP22-induced slope decrease



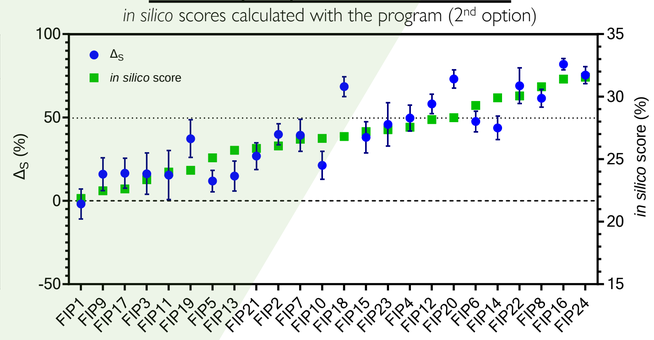
Comparison of existing methods with IFC

	HPLC	Fluorescence	**IFC**
Precision	+ +	-	+
Analysis duration	- -	+ +	+ +
Costs	- -	- -	+ +
Suitable for large libraries	- -	-	+ +

Mca-Dpa cleavage (MMP-13) : Fluorescence **increases**.
Mca-Dpa cleavage with interferent peptide : slope (hydrolysis rate) **decreases**.

Δ_S = % slope decrease with respect to substrate alone, ranked by sequence *in silico* scores

in silico scores calculated with the program (2nd option)



The IFC efficiency **increases** with the *in silico* score

2.b IFC : Selectivity

in silico score for chosen sequences and for every protease involved

in silico scores calculated with the program (3rd option).

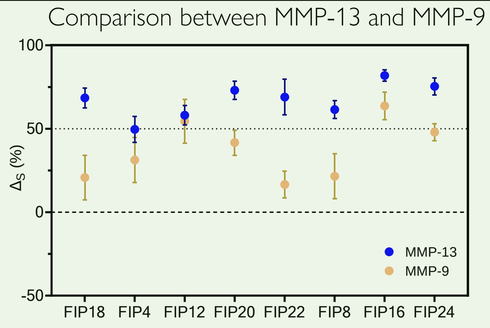
Sequence number	MMP-1	MMP-2	MMP-3	MMP-9	MMP-13	ADAMTS-4	ADAMTS-5
18	15.3	10.5	9.5	19.6	26.8	9.7	8.6
4	14.8	10.4	10.6	19.7	27.5	9.0	8.9
12	14.7	10.0	10.6	21.1	28.2	9.3	9.1
20	16.4	10.4	11.0	20.6	28.3	10.7	10.1
22	18.3	15.0	11.5	22.5	30.1	11.6	10.9
8	17.8	15.0	12.6	22.6	30.8	10.9	11.1
16	17.7	14.6	12.6	23.9	31.4	11.2	11.3
24	19.4	15.0	13.1	23.4	31.5	12.5	12.4

0% 10% 20% 30% 40% 50%

MMP-9 **most efficient protease** after MMP-13

Slope difference as a percentage of the Substrate slope

Comparison between MMP-13 and MMP-9



All peptides are **selective** towards MMP-13

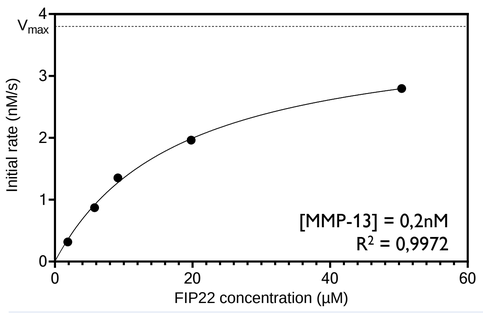
Calculated selectivity of the 8 chosen peptides

FIP	$\Delta_{S,\ MMP\text{-}13}$ (%)	$\Delta_{S,\ MMP\text{-}9}$ (%)	Selectivity
18	68,5	20,8	3,29
4	49,7	31,3	1,59
12	58,1	54,5	1,07
20	73,0	41,7	1,75
22	69,0	16,6	4,16
8	61,5	21,5	2,86
16	81,9	63,7	1,29
24	75,4	47,9	1,57

FIP12 and FIP16 : **poor selectivity**
Probably due to **proline** at **P2** position
(higher MEROPS value for MMP-9 than MMP-13)

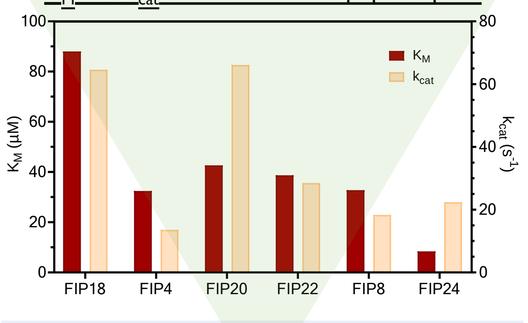
3 UHPLC Verification

Michaelis-Menten fit for FIP22



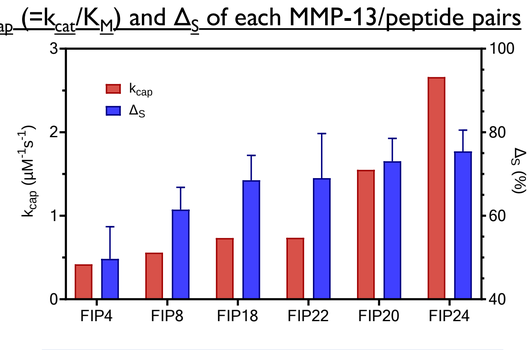
Kinetic reactions with **increasing** [FIPx] at a **fixed** [MMP-13]

K_M and k_{cat} of each MMP-13/peptide pairs



P1' position : **Leu** lowers more K_M than **Met**
P2' position : **Arg** lowers more K_M than **Asn**

k_{cap} (=k_{cat}/K_M) and Δ_S of each MMP-13/peptide pairs



The IFC efficiency **increases** with the k_{cap} calculated with UPLC

Conclusion and perspectives

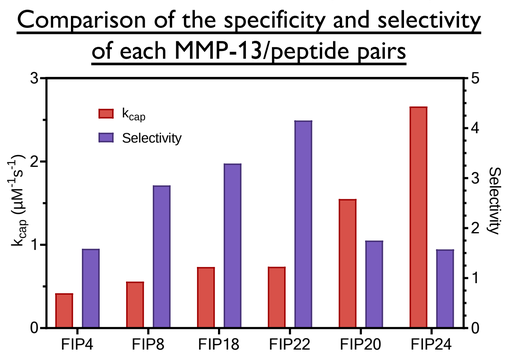
Selective and **efficient** methodology to screen **protease-sensitive peptides :**

Program predicting the **sequence library** to screen

New screening method that is **faster**, **cheaper** and **accurate** to rank peptides

Usable for **various projects**, depending on the specifications

Comparison of the specificity and selectivity of each MMP-13/peptide pairs



Use of **IP22** for the main project
(the most selective one, with good efficiency) :
- **click chemistry** to the carrier;
- **thiol-Michael addition** to the siRNA lipoplexe.

Proof of method robustness on other enzymes complexes.
Research on the **theorical connection** between Δ_S and k_{cap}.

References

Global Burden of 369 Diseases and Injuries in 204 Countries and Territories, 1990–2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *The Lancet* **2020**, *396* (10258), 1204–1222.

Scotti, C. *et al.*, Recapitulation of Endochondral Bone Formation Using Human Adult Mesenchymal Stem Cells as a Paradigm for Developmental Engineering. *Proceedings of the National Academy of Sciences* **2010**, 107 (16), 7251–7256.

Mathieu, M. *et al.*, Induction of Mesenchymal Stem Cell Differentiation and Cartilage Formation by Cross-Linker-Free Collagen Microspheres. *Eur Cell Mater* **2014**, *28*, 82–96; discussion 96-97.

Stay tuned for the publication

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