FLEXOSOME

Placing enzymes where they should be

MOTIVATION

Nature contains a huge variety of environments and organisms. The various metabolism and huge amount of enzymes which are now being discovered thanks to the metagenomic approach are our inspiration.
For us those organisms are a tool box which synthetic biology enables us to combine.
To wield and combine these tools we designed a customisable, multipurpose complex.

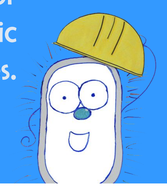
INTRODUCTION

Cellulosomes from anaerobic cellulolytic organisms degrade cellulosic substrates more efficiently. In a cellulosome different enzymes are assembled into a scaffoldin base via dockerin-cohesin domains.
Inspired by the advantages of the modular design of the cellulosome, other researchers have tried to produce artificial multi-enzymatic constructs utilising nanomolecules, nucleic acid-protein conjugates and streptavidin-biotin systems as scaffolds.
However, the dockerin-cohesin interaction has been proven to be nearly half the strength of a covalent bond, making it ideal for our multi-functional tool: the "Flexosome".
This combines the scaffoldin protein from cellulolytic bacteria with varying and exchangeable enzymes.

OBJECTIVES

Create a protein construct able to ensure a high local concentration of enzymes and exhibit different enzymatic functions.

The construct will be fully customisable for single and multi-step enzymatic processes.



1. The scaffoldins

Synthetic scaffoldins with cohesin domains from *Acetivibrio cellulolyticus*, *Bacteroides cellulosolvens*, *Clostridium cellulolyticum* and *Clostridium thermocellum* were created with ***A. cellulolyticus*** and ***C. thermocellum*** scaffoldins as a basis.

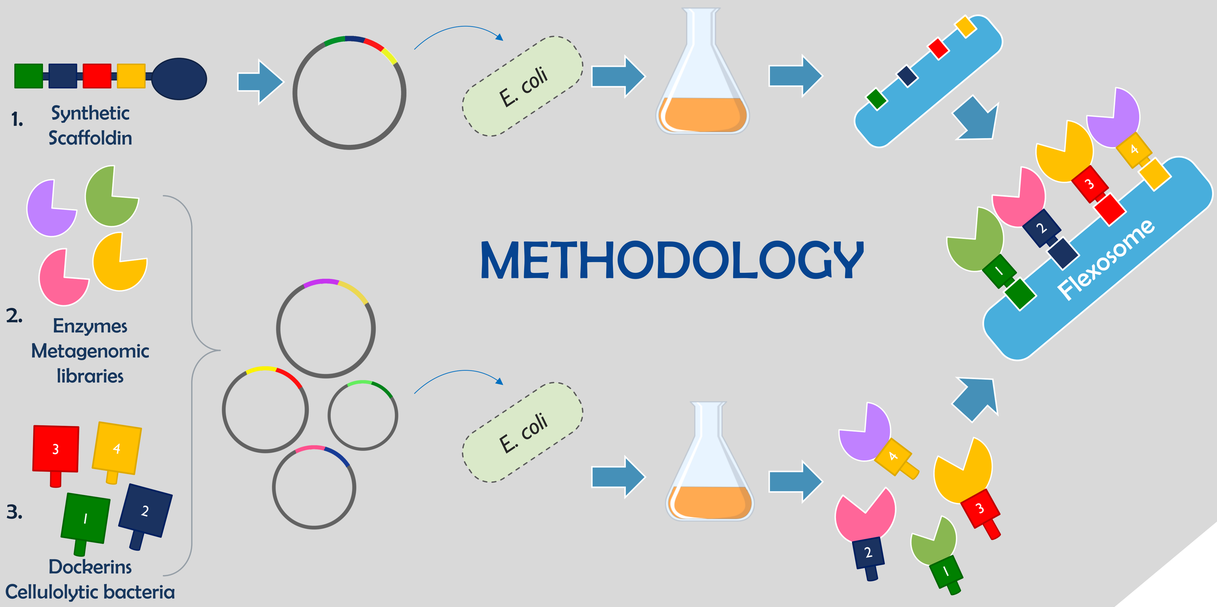
2. The enzymes

Phosphatase, esterase and cellulase were selected from metagenomic libraries and RFP was incorporated as a reporter. Each protein can easily be verified.

3. The dockerins

Each dockerin binds to a different cohesin domain of our synthetic scaffoldins.

METHODOLOGY



4. The method

The scaffoldin was synthesised and every enzyme fragment was ligated to a dockerin. All constructs were transformed into *E. coli.* Both the enzyme-dockerins and scaffoldins were expressed and isolated by His-Tag purification and size exclusion chromatography in a FPLC system.

APPLICATIONS

The Flexosome has a great potential for the enhancement of new enzymatic combinations or already existing processes.

Industry
Optimise enzymatic cascades

Pharma
Synthesis of stereospecific drugs

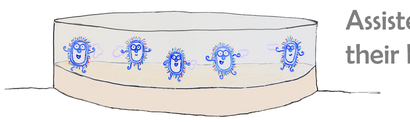
Environment
Multistep degradation of pollutants

PARTS SUBMITTED

BBa_K1865000: A dockerin derived from *B. Cellulolyticus* (BCEL).
BBa_K1865001: The eforRED biobrick fused to BCEL.

COLLABORATIONS

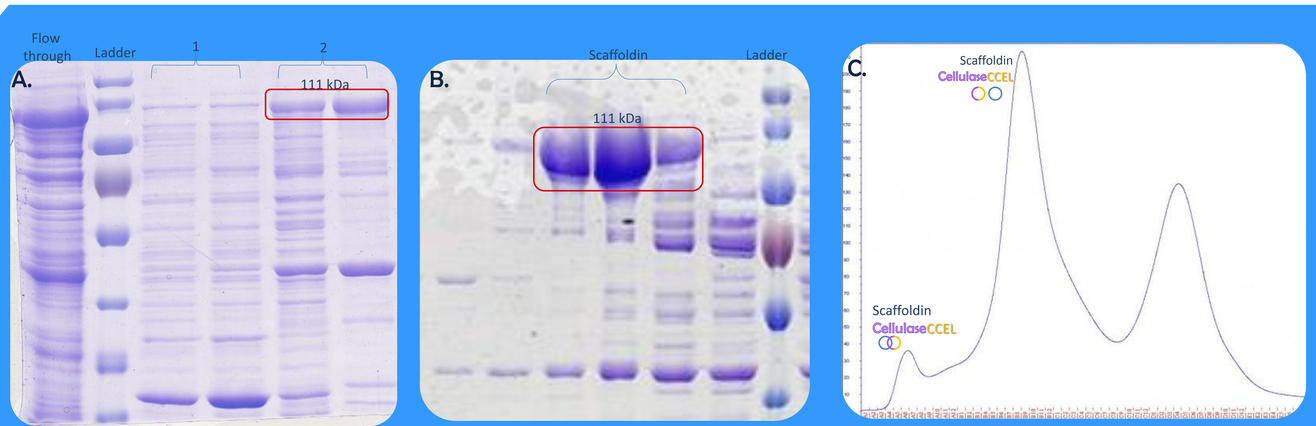
Aachen enzymes were fused with dockerins as a first application of our Flexosome.



Assisted Aix-Marseille with their human practices.

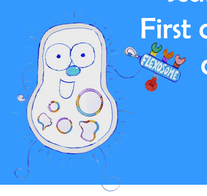
RESULTS

A. SDS gel of the two His-Tag purification peaks. The bands of 111kDa correspond to the scaffoldin
B. SDS gel after size exclusion chromatography (SEC) of the scaffoldin fractions.
C. SEC chromatogram indicating interaction between our scaffoldin and the CellulaseCCEL construct.



CONCLUSIONS

Co-purification will be continued until more representative results are gained. Further activity tests will have to be developed to accurately proof our results.
We constructed, expressed and purified both scaffoldins, CellulaseCCEL and RFPACEL.
First co-purification experiments indicated an interaction between the scaffoldin and dockerin constructions.



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References
Chen R., Chen Q., Kim, H., Siu K.-H., Sun, Q., Tsai, S.-L., Chen W. (2014) Biomolecular scaffolds for enhanced signaling and catalytic efficiency. *Current Opinion in Biotechnology.* Volume 28, Pp. 59 – 68
Hirakawa, H. Haga, T., Nagamune, T. (2012) Artificial Protein Complexes for Biocatalysis. *Topics in Catalysis.* Volume 55, Pp. 1124 –1137
Hyeon, J. E., Jeon, S. D., Han, S. O. (2013) Cellulosome-based, Clostridium-derived multi-functional enzyme complexes for advanced biotechnology tool development: advances and applications. *Biotechnology Advances.* Volume 31, Pp. 936 – 944

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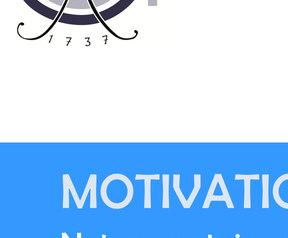

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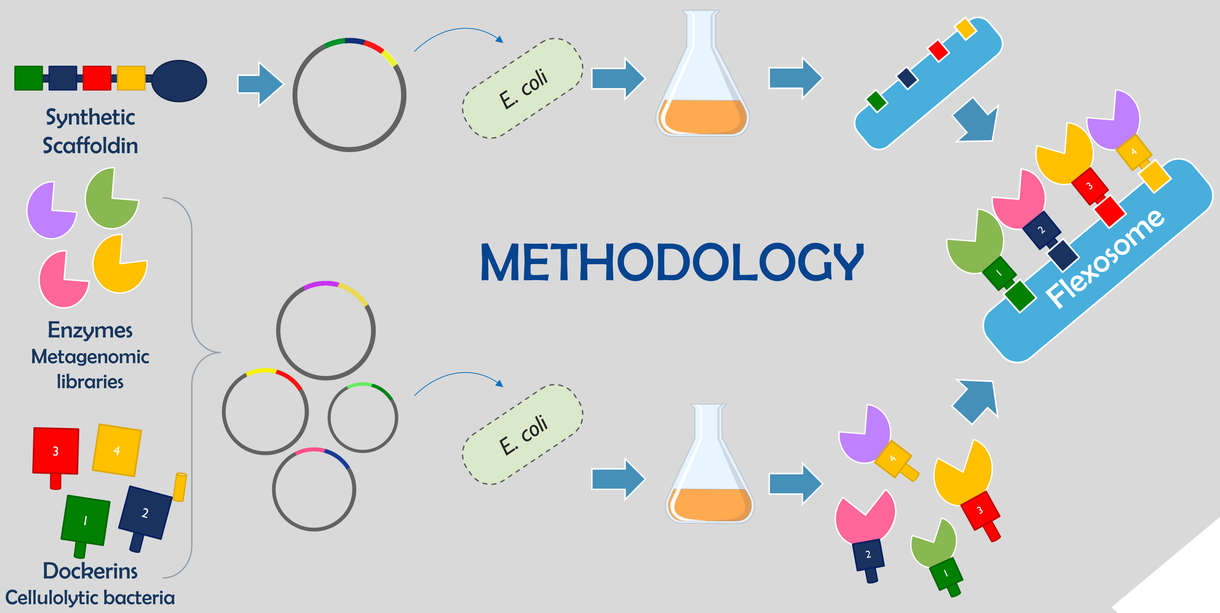

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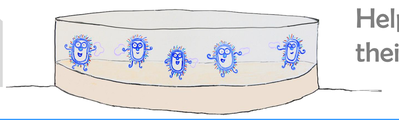

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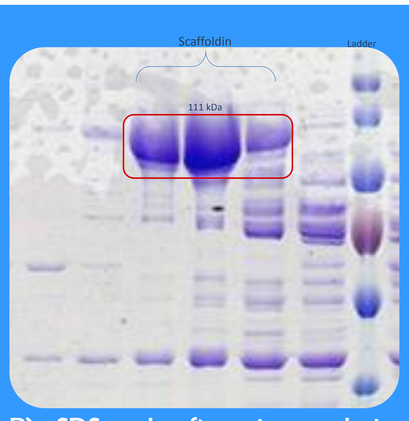

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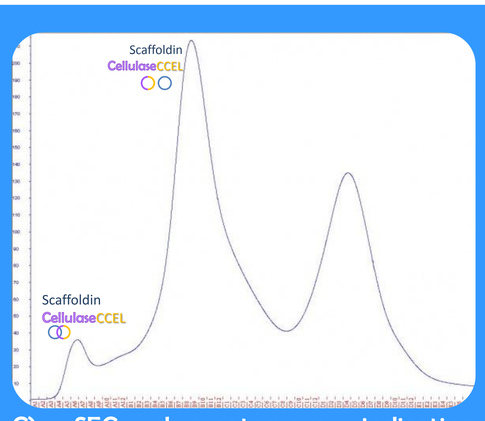

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