Exploring Non-Canonical Amino Acid Integration in Fibroblast Growth Factors

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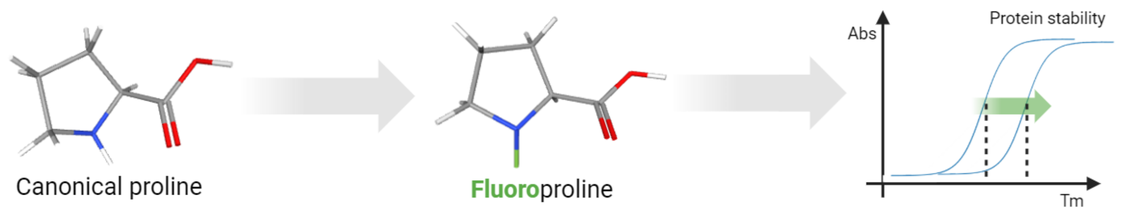
BACKGROUND

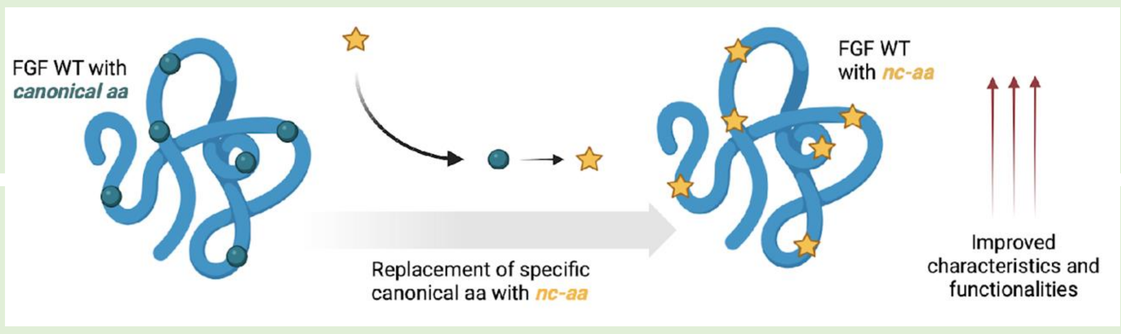
Fibroblast Growth Factors (FGFs) regulate vital mechanisms in complex organisms, including cell migration, proliferation, differentiation, and survival. Their potential for ***wound healing, cancer treatment***, and ***tissue repair*** has attracted significant interest.

Expanding the genetic code enables the use of ***non-canonical amino acids*** (ncAAs), absent in the human genome. Robust methods for genetic code expansion facilitate the incorporation of ncAAs into protein polypeptide chains. This enhances ***stability*** and ***activity*** by introducing ***novel functionalities*** and ***chemical properties*** into enzymes [1].



AIM

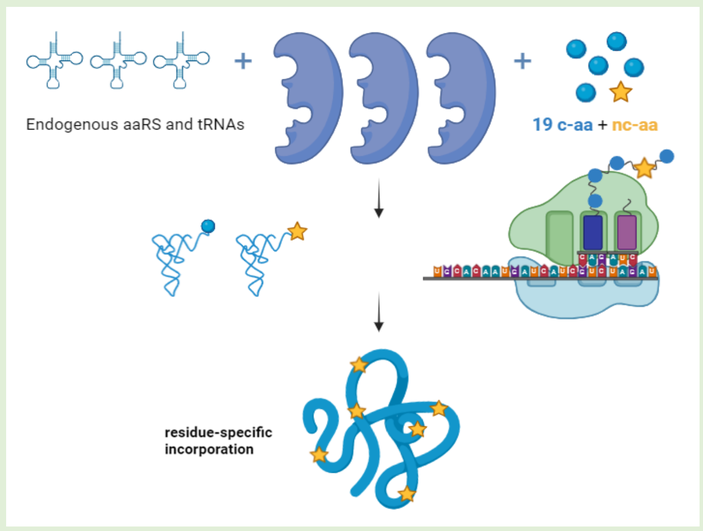
Using the ***SPI method***, we aim to assess how the insertion of ncAAs affect the ***stability*** and ***activity*** of FGFs proteins and to understand the ***general applicability*** of nc-aa incorporation to improve FGF proteins.



In the ***SCS method***, we introduce ncAAs at specific positions within the protein. These ncAAs serve as handles for click reactions, enabling the ***immobilization*** or ***interaction*** of FGFs with other ***biological entities***.

METHODS

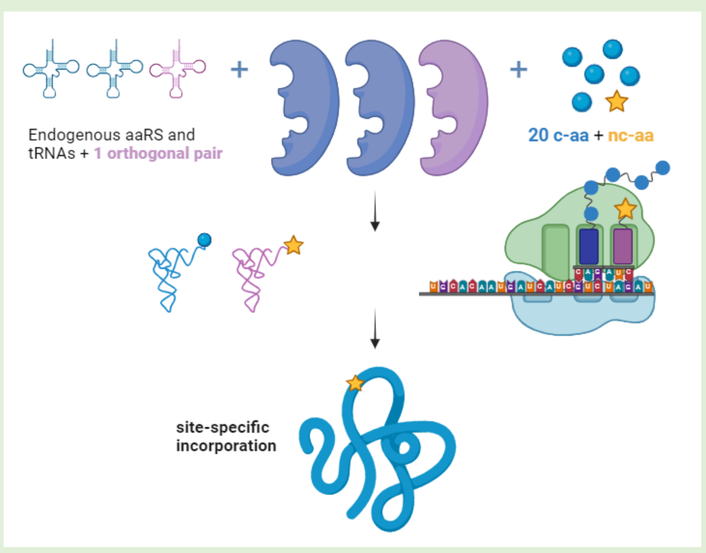
Two state-of-the-art methods will be used: Selective Pressure Incorporation (**SPI**) and Stop Codon Suppression (**SCS**).



SPI

Global replacement of cAAs with the corresponding ncAAs with ***residue-specific incorporation.*** [2]

- It requires an ***auxotrophic system*** to replace the cAAs ;
- It employs ***native expression systems*** readily available in the organism;
- It allows the simultaneous incorporation of multiple ncAAs into the same protein;
- SPI ***does not increase*** the size of the ***amino acid alphabet***: cAA → ncAA;
- It uses a pool of ncAA similar to the 20 cAA.



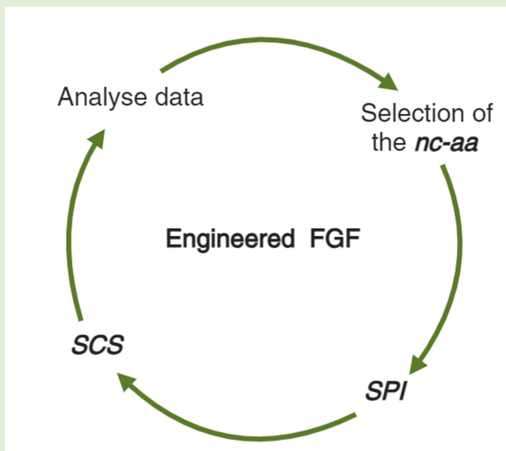
SCS

Replacement of a specific natural amino acid with a nc-aa through ***site-specific incorporation.*** *[2]*

- It does ***not require*** an ***auxotrophic system*** to replace the cAAs;
- SCS uses ***non-native expression systems***;
- SCS the ***ncAAs*** are ***added to*** the ***20 cAA*** repertoire;
- SCS allows the site-specific incorporation of ncAAs at virtually any position;
- SCS allows the incorporation of a variety of surrogates distinctly different from the 20 cAAs.

WORKFLOW

- Selection of the FGFs of interest along with the ***appropriate nc-aa***;
- ***SPI*** approach to provide a general overview of the critical sites and amino acids within FGF structures;
- ***SCS*** approach to the replacement of specific amino acids for ***immobilisation*** or ***functionalisation***;
- Analysis of collected data to understand ***future implications*** for industrial applications and directions to optimize FGFs with nc-aas.



CURRENT STATE

After completing a training period, I started my doctoral project with the SPI method. I decided to start with 4-fluoroproline and difluoroproline and an E. coli auxotroph strain. Following this, I made the cells competent, enabling them to efficiently uptake and express FGF2, FGF4, and FGF8 plasmids, all of interest to our company. [3]

Currently, my focus lies on optimizing the SPI technique to ensure the successful integration of non-canonical amino acids within the cells, a crucial step for enhancing FGF production.

In June, I begin a four-month research visit to Professor Budisa's lab in Canada. His expertise in the field is renowned. I aim to learn new techniques to advance my work at Enantis

REFERENCES

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