A *de novo* enzyme catalyzes a life-sustaining reaction in *Escherichia coli*

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Contents

Introduction

- **One key goal of synthetic biology is to produce novel enzymes that are catalytically active *in vitro* and biologically functional *in vivo***
- **Development of artificial enzymes with new functions and ability to catalyse non-natural reactions could be very valuable in synthetic biology applications**
- **De novo proteins**
  - Created from artificial sequences designed in laboratory
  - Have been used to regulate gene expression but achieving life-sustaining functions has been more challenging
- **This Study**
  - Binary patterning to create combinatorial libraries of de novo sequences to discover new proteins with chemically and biologically important functions
  - Create proteins that could replace the function of natural enzyme and rescue the deletion of fes (ability to grow on iron-limited media)
  - Deletion of essential gene, introducing artificial gene for sustaining viability

Syn-F4

- **formed via random mutagenesis and directed evolution from Syn-IF**
- **is catalytically active both *in vivo* and *in vitro***

- **Δfes cells**
  - in previous study, no hydrolytic activity in vitro -> now further improvements
  - cannot grow in iron-limited environments
  - encodes enzyme called ferric enterobactin (FeEnt) esterase

- **Syn-F4 hydrolyzes the siderophore ferric enterobactin (FeEnt)**

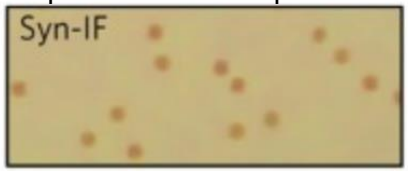
  → enantioselectivity

Hydrolysis of ferric enterobactin (FeEnt)

Colonies in iron-rich medium

The evolved *de novo* protein

The parental *de novo* protein



Native *E.coli* protein

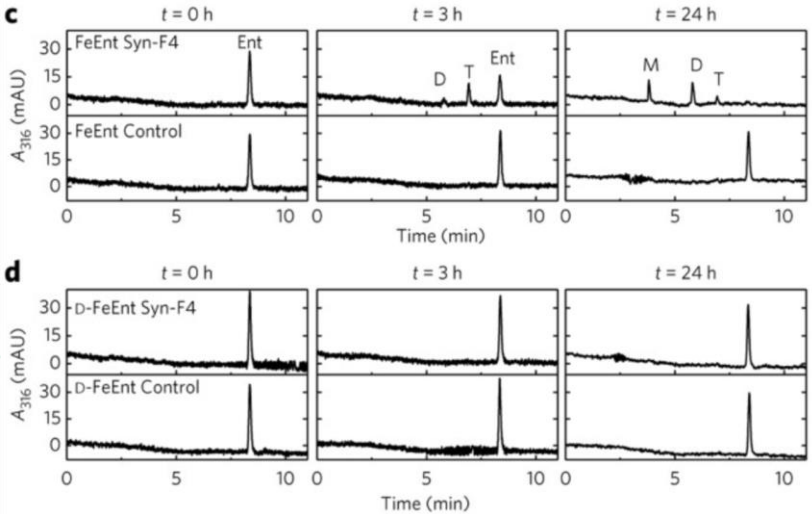
Red = Slow growth, not able to hydrolyze the FeEnt siderophore

White = Robust growth, able to degrade FeEnt

Enantioselectivity

c) Syn-F4 hydrolyzes natural FeEnt L-enantiomer

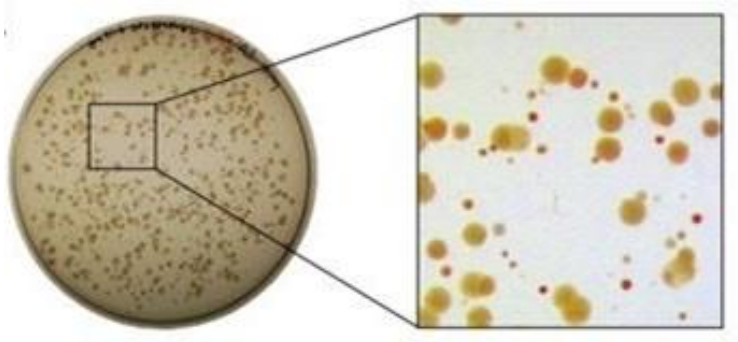
d) No hydrolysis on non-natural D-enantiomer



T = trimer
D = dimer
M = monomer

Determination of structure and active site of Syn-F4

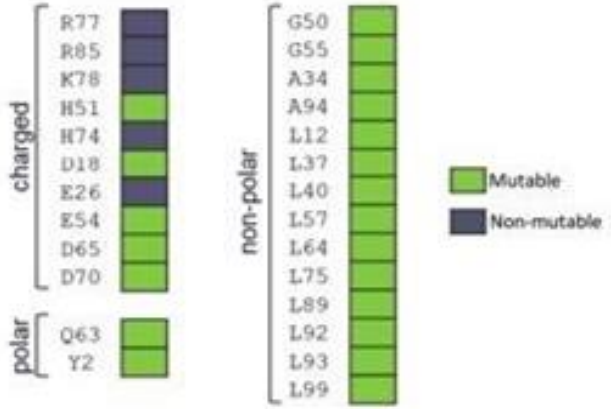
- **Standard approaches were not suitable**
  - low sequence similarity, unstable structure

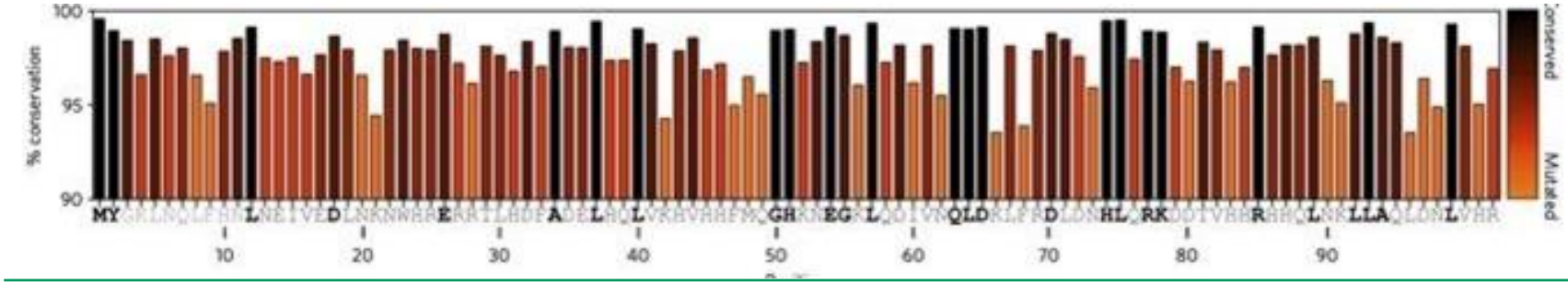


- **Solution: mutagenesis, high-throughput sequencing and computational methods**
  - error-prone PCR → 1-3 residue differences
  - amino acid conservation at residues essential for enzymatic function

Determination of structure and active site of Syn-F4

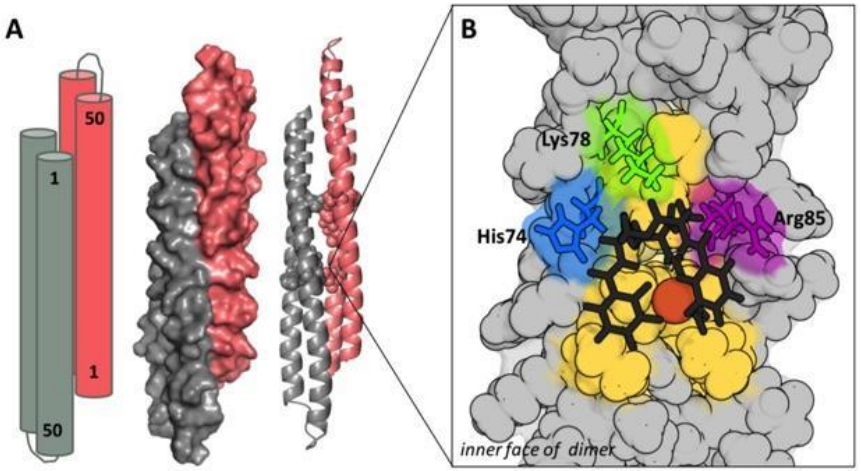
- **27 highly conserved residues**
  - 15 non-polar residues: structural function
  - saturation mutagenesis → 5 charged residues essential





Structure and active site of Syn-F4

- By gel-filtration analysis Syn-F4 forms a dimer
- Computational structure prediction
  - Predicted lowest energy dimer structure
  - Predicted binding site of FeEnt
  - The conserved catalytic residues
- Predicted structure differs from the natural enzyme



Challenges

- **Determining a structure of Syn-F4 by traditional methods was unsuccessful**
  - Structure of protein is dynamic and equilibrates between monomeric and dimeric a-structures → NMR and crystallography not able to capture structures
- **De novo proteins can not be compared to the natural ones → sequences are very different!**
  - This is maybe the most important overall challenge with de novo proteins and conventional methods in finding what in the protein is important for the catalytic activity (however, using other methods described previously, good results were achieved)
- **Novel Enzymes**
  - Low levels of activity
  - May require cofactors, but which?
  - The Syn protein may function by a different mechanism than the deleted protein, and novel activities would not be detected in experiments designed to assay the natural enzyme

Results

- **Syn-F4 is the first de novo enzyme that works both *in vitro* and *in vivo***
- **Genetic rescue (observed as growth on minimal medium) correlated with enzymatic activity (observed as color of colony) in vivo**
- **When *fes* gene was replaced with synthetic gene encoding Syn-F4, colonies formed but slower than from a plasmid**
    - Chromosomal incorporation of a gene encoding a novel enzyme → Life-sustaining semi-artificial genomes
- **Combinatorial libraries of de novo sequences can provide rich sources of diversity for the discovery of new proteins with chemically and biologically important functions**
- **Demonstrates that novel proteins can provide life-sustaining enzymatic functions in living organisms and have similar functions as natural enzymes**
    - Without need for computer design or billions of years of evolution

Importance and Path Forward

- **Chemical rules of nature and billions of years of evolution no longer limitation**
  - Toolkit for synthetic biology not limited to genes and proteins that already exist in nature
- **New solutions for biological challenges**
- **Creation of other de novo proteins performing important biological roles in cells**
  - Already new research available (a de novo peroxidase)
- **High value for industrial biotechnology applications that relies on enzymes**
- **Construction of artificial genomes capable of sustaining life**

References

Donnelly, A., Murphy, G., Digianantonio, K. *et al.* A *de novo* enzyme catalyzes a life-sustaining reaction in *Escherichia coli*. *Nat Chem Biol* 14, 253–255 (2018). https://doi.org/10.1038/nchembio.2550

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Thank you!
Questions?