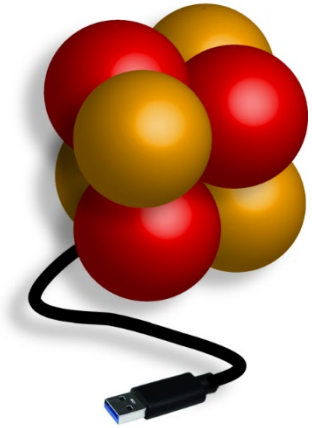
ERA CoBioTech

BIOTECH RESEARCH AND INNOVATION HACK 2021

Final seminar of the cofunded projects of ERA CoBioTech

Electric plug adapters for iron-sulfur enzymes: exploiting Nature's catalytic potential for biotechnology



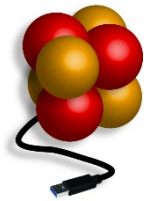
IRONPLUGNPLAY

Greg Bokinsky, Bionanoscience Department,
TU Delft, The Netherlands

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

29.09.2021

Project partners



CSIC Madrid
- Miguel Alcalde
- Eva Garcia Ruiz

Pasteur Institute
- Frederic Barras
- Simonetta Gribaldo
- Francesca D'Angelo
- Pierre Garcia

CNRS Grenoble
- Sandrine Ollagnier de Choudens
- Martin Pelosse

CNRS Marseille (affiliated)
- Beatrice Py

TU Delft
- Greg Bokinsky
- Elena Fueyo
- Helena Shomar
- Rita Robelo

Isobionics B.V. (industrial partner)
- Frank Fluitman
- Matthew Styles
- Georg Lentzen

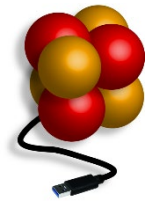
Budget: €850,000

Dates:

01 June 2018 – ~~31 May 2021~~ 31 -03-2022

(corona-related extension)

Making useful products with engineered microbes



Artemisinin biosynthetic pathway from wormwood plant

gene transfer

Antibiotic pathways from uncultivatable organisms

Nitrogen-fixing enzymes

foreign enzymes

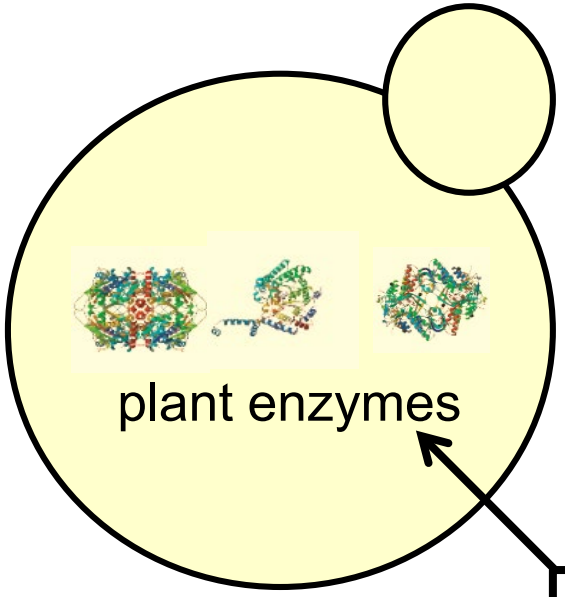
Yeast or *E. coli* or anything that grows quickly

Affordable anti-malaria treatment

Novel antibiotics

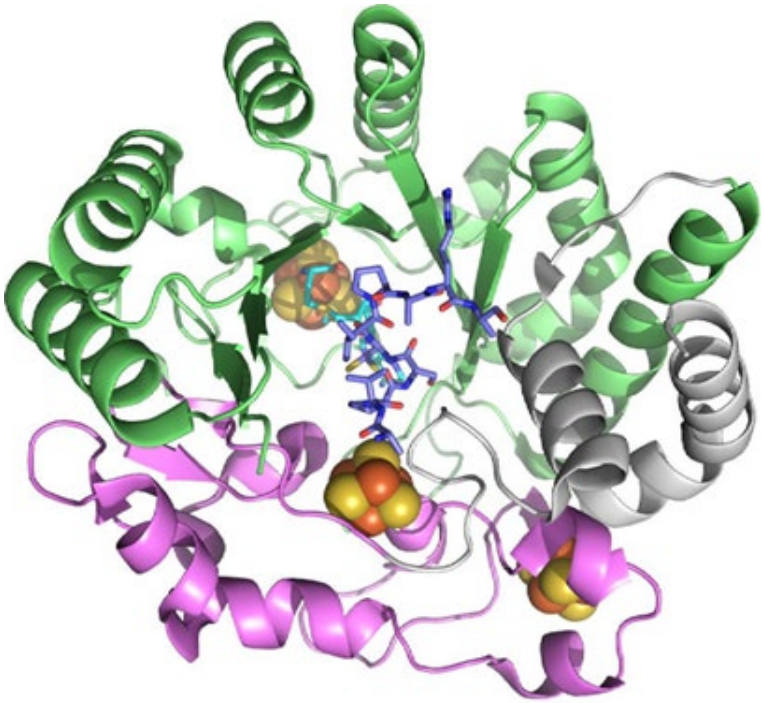
“Green fertilizers”

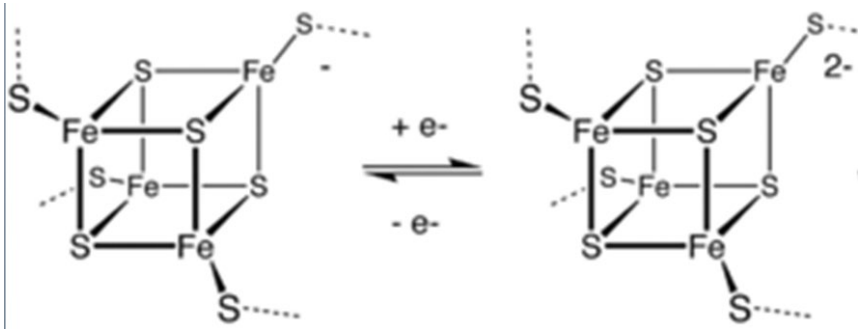
Making useful products with engineered microbes



What if these foreign enzymes don't function in their new host?

Expressing iron-sulfur enzymes in new hosts is scary for microbial engineers



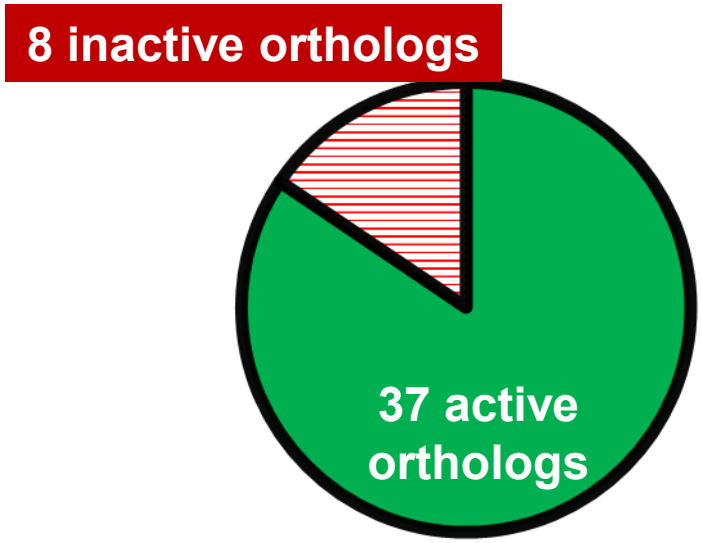


Required for methyl transfer reactions, reduction reactions, sulfur insertion, epoxidation, hydroxylation…

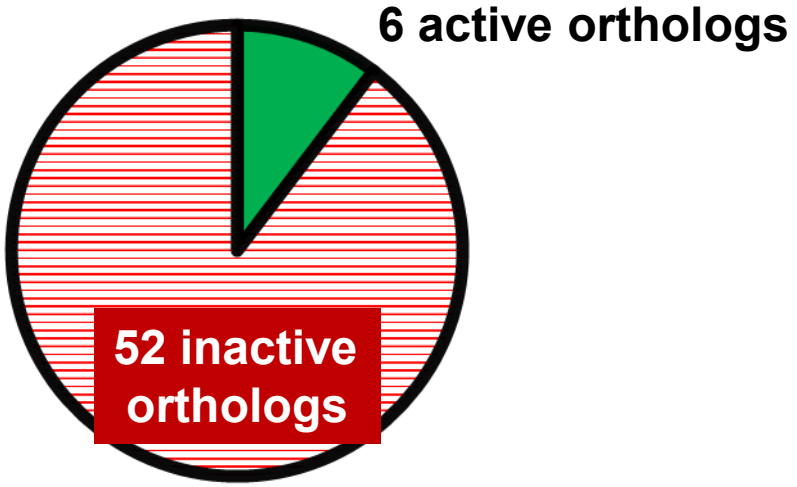
Biosynthesis of: fragrances, antibiotics, vitamins, anti-cancer compounds, biofuels, fixed nitrogen

Fe-S enzymes are less compatible between species than non-Fe-S enzymes

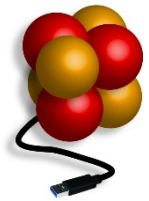
Activity of non-Fe-S enzyme orthologs in *E. coli*



Activities of Fe-S enzyme orthologs in *E. coli*

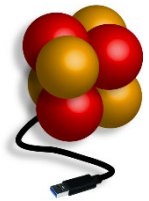


Introduction



- **Project objectives:** to identify and overcome barriers to using heterologous iron-sulfur enzymes in engineered biosynthetic pathways.

- **Scientific approach:** We used techniques from:
  - **Synthetic biology**
  - **Metabolic engineering**
  - **Iron-sulphur biochemistry**
  - **Bioinformatics**
  - **Directed evolution**



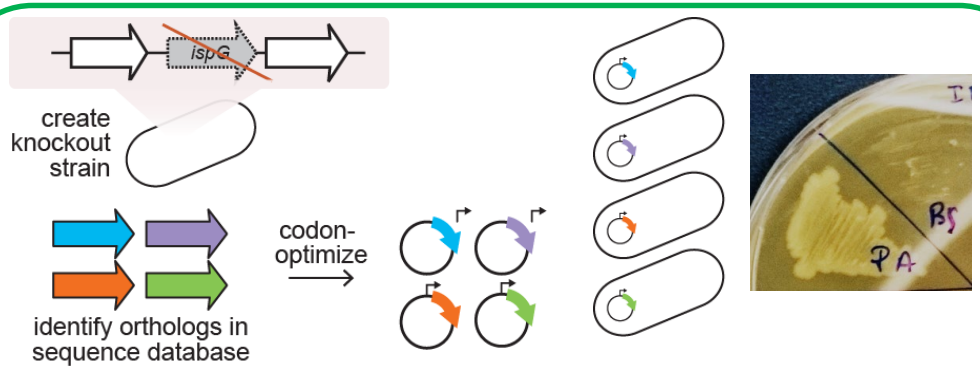
Finding plug adapters for iron-sulfur enzymes

1) **Which Fe-S enzymes are incompatible with common hosts for biotechnology?**
   Mapping the limits of inter-species compatibility of FeS enzymes

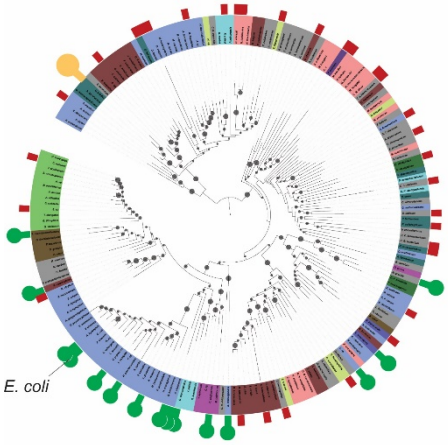
2) **What's the cause of the apparent incompatibility?**
   Identifying and overcoming the obstacles to FeS enzyme compatibility

3) **What can we do about it?**
   Applying lessons learned to interesting biosynthetic pathways

What we did, in summary

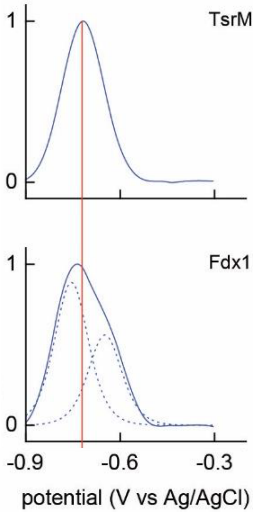


Mapping compatibilities with a growth screen

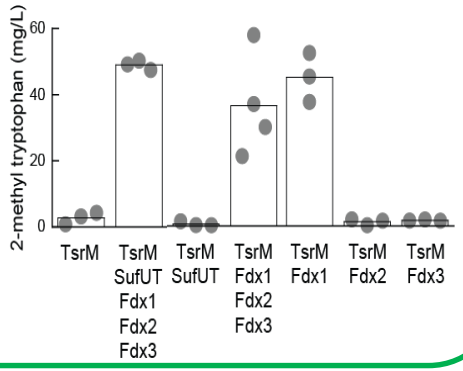


Identifying phylogenetic compatibility range of various iron-sulphur enzymes

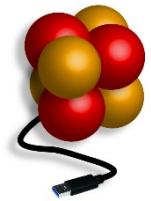
Identifying the biochemical basis of compatibility requirements

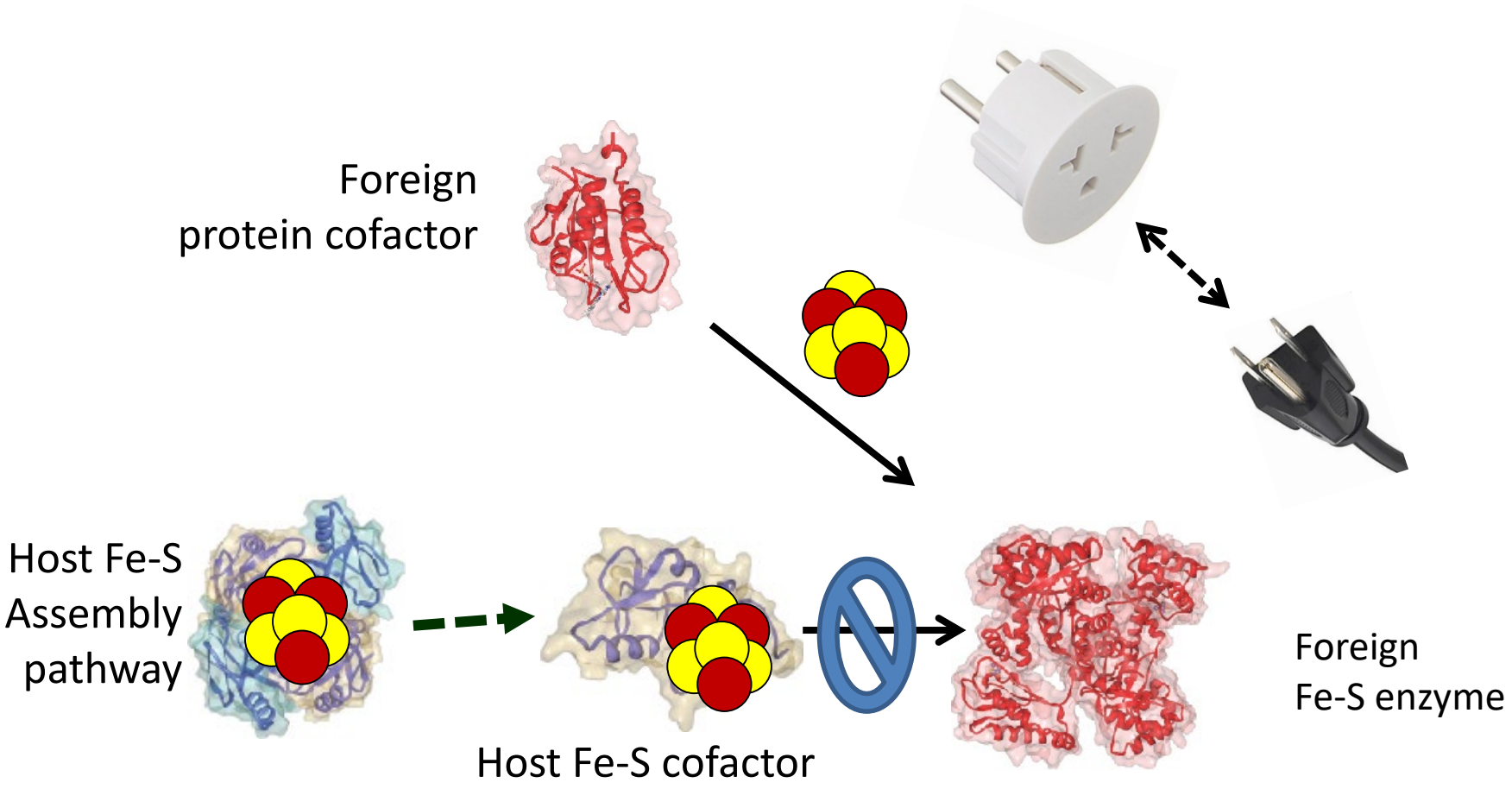


Putting our findings to work: **Making useful compounds**

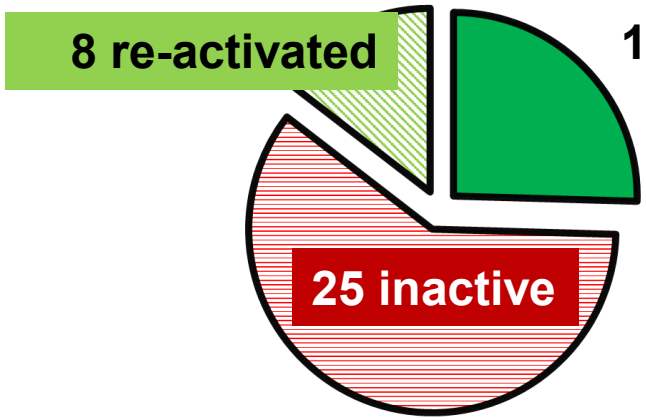


Finding protein plug adapters for foreign Fe-S enzymes

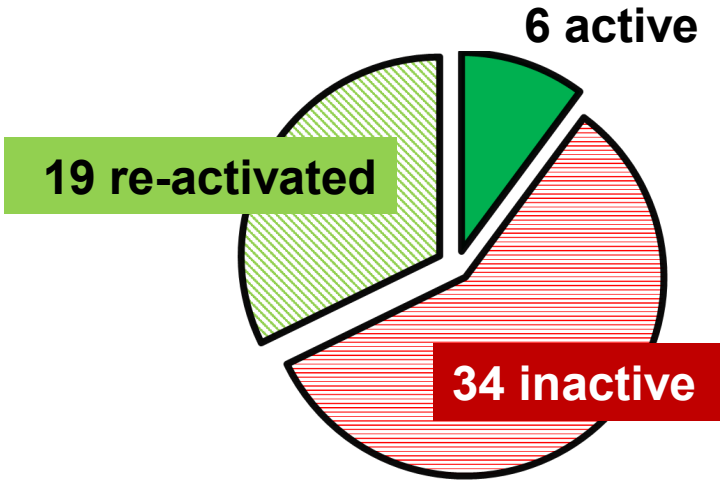




What we learned

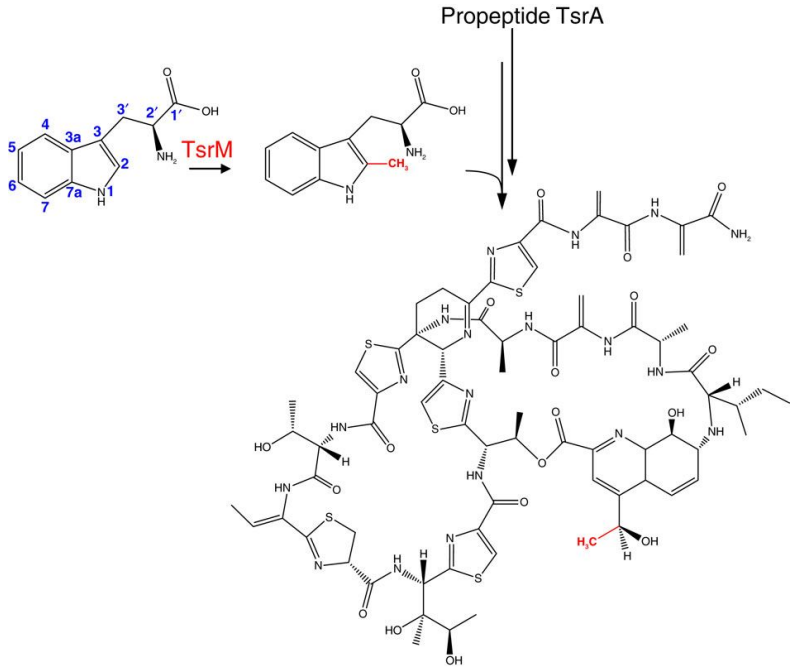


Some Fe-S enzymes need specific maturation pathways for the Fe-S cluster:
Compatibility depends upon phylogenetic distance…

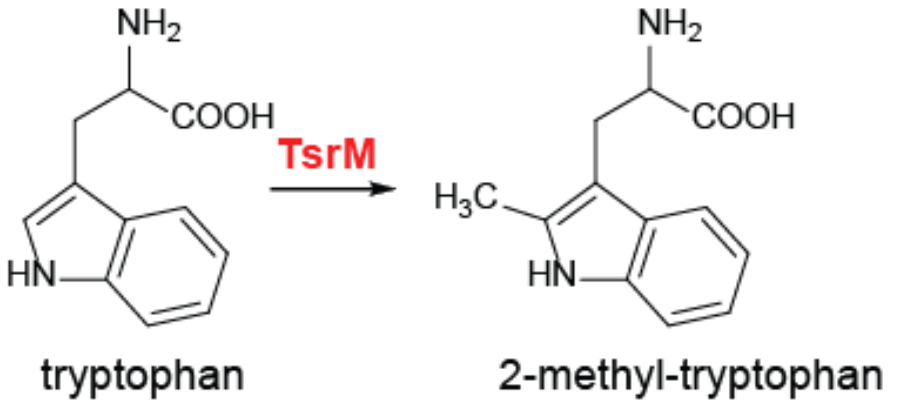


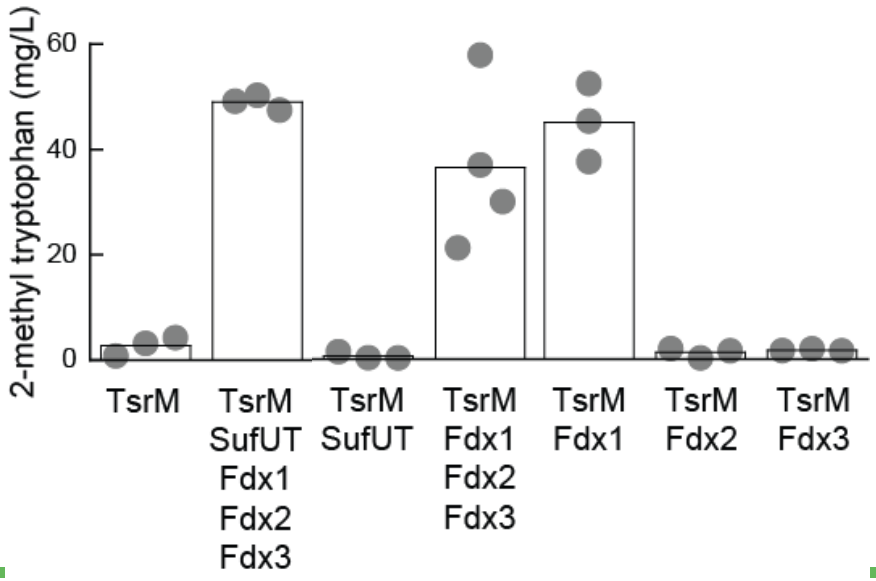
But many more enzymes require specific "electric plugs" – i.e. electron carriers.
<u>This is the main problem with Fe-S enzymes.</u>

Putting our findings to use

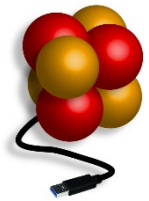


Thiostrepton
(from *Streptomyces laurentii)*





Technology Transfer and market potential

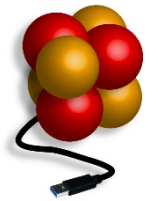


- ***Main lesson:*** this turned out to be much more of a basic research project, rather than an applied project.

- **We have better defined the separate contribution of several bottlenecks and have found ways to solve them.**
  - **Specific protein cofactors** have been identified that can be co-expressed in host organisms --> these will be shared

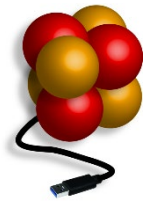
- We found a “plug adapter” interesting for our industrial partner: still evaluating results with our partner and how best to use the finding

Technical overview / RRI aspects



- ***Data storage & sharing*** *between consortium members via SURFdrive (with mixed results)* – some lapses back to emailing manuscript files
- *Consortium-wide agreement before moving towards publication to ensure no disclosure of patentable IP (as per Consortium Agreement)*
- ***Open science:*** *posting work to biorxiv pre-print server while manuscripts are under review at an open-access journal*
- ***Plug adapter parts*** *will be shared freely upon publication via plasmid sharing non-profit (AddGene)*

Summary

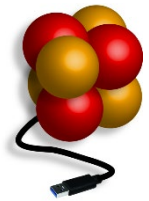


- ***We proposed*** *to find and overcome obstacles to the use of iron-sulfur cluster enzymes in engineered biosynthetic pathways.*
  - *The focus was on making* ***inactive enzymes active,*** *not on* ***increasing enzyme activity.***

- ***We discovered*** *specific cofactors necessary for iron-sulfur enzyme activity in engineered hosts –* ***turned out to not be what we expected, a useful discovery.***
  - We've brought this area of research from "anecdotes" to "useable design principles."
  - We've identified specific cofactors / pathways needed for heterologous Fe -S enzymes

- **Future projects:**
  - Identifying which "electric plug adapter proteins" match with specific Fe -S enzymes in a given genome
  - Changing the plug adapter specificity for Fe-S enzymes
  - **Testing our design principles by** applying our findings to a biosynthetic pathway where the Fe-S enzyme is the bottleneck

Project outcomes



New Results

Follow this preprint

Resolving phylogenetic and biochemical barriers to functional expression of heterologous iron-sulphur cluster enzymes

Helena Shomar, Pierre Simon Garcia, Elena Fernández-Fueyo, Francesca D'Angelo, Martin Pelosse, Rita Rebelo Manuel, Ferhat Büke, Siyi Liu, Niels van den Broek, Nicolas Duraffourg, Carol de Ram, Martin Pabst, Simonetta Gribaldo, Beatrice Py, Sandrine Ollagnier de Choudens, Gregory Bokinsky, Frédéric Barras

doi: https://doi.org/10.1101/2021.02.02.429153

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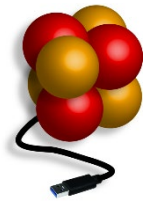
(preparing revision for an open-access journal)

- *Invited talks* (biotechnology societies, Fe-S and electron transfer conferences, industry (Google))
- *We've received requests for collaborations with external biotech companies*

General Evaluation



- **Laying the groundwork for systematic exploitation of Fe-S enzymes:** we've brought the Fe-S synthetic biology from anecdote-based knowledge to an engineering field

- This research was very useful for linking basic scientists who do not typically consider the practical applications of their research

- **A big thank-you to the ERACoBioTech team** for enabling the COVID extensions – we could not have done this without you

Contact details

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