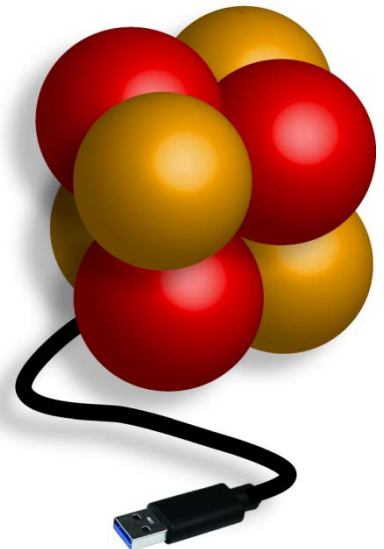


“Biotechnology for a sustainable bioeconomy”

Activating catalysts for metabolic engineering:  
Portable adapters for iron-sulfur enzymes

IRONPLUGNPLAY

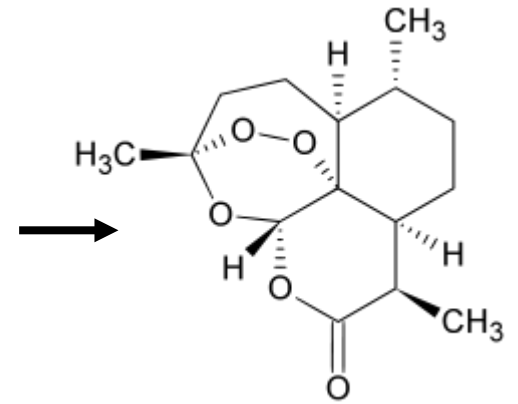
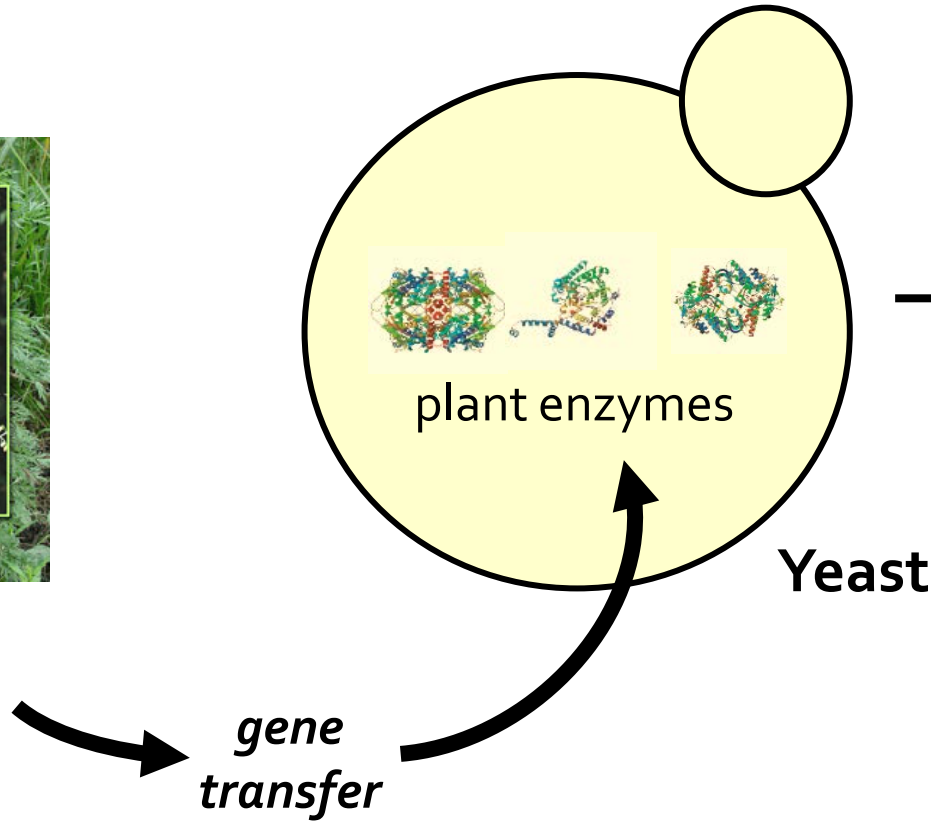
Greg Bokinsky, TU Delft



# How can we turn microbes into chemical factories?



Wormwood plant

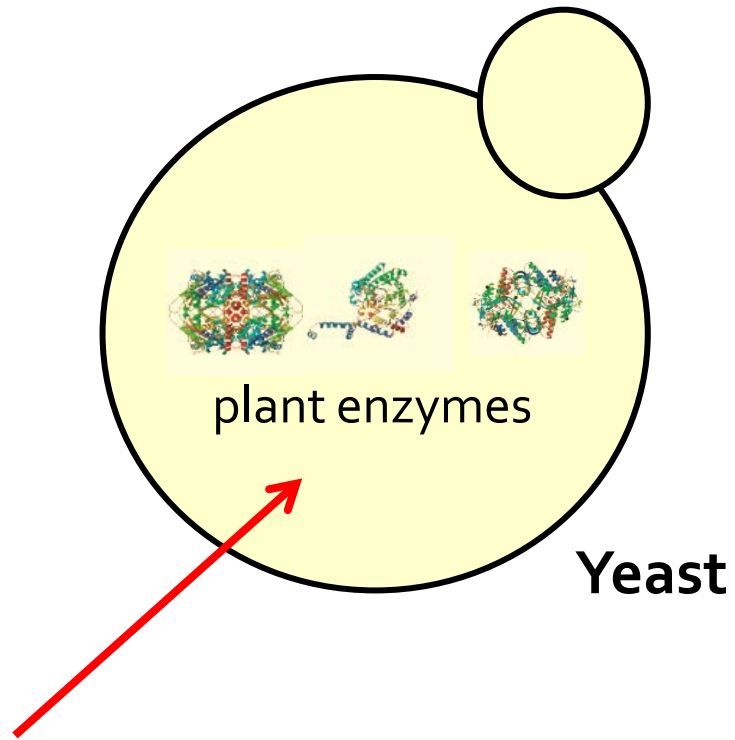


Artemisinin

(anti-malarial)

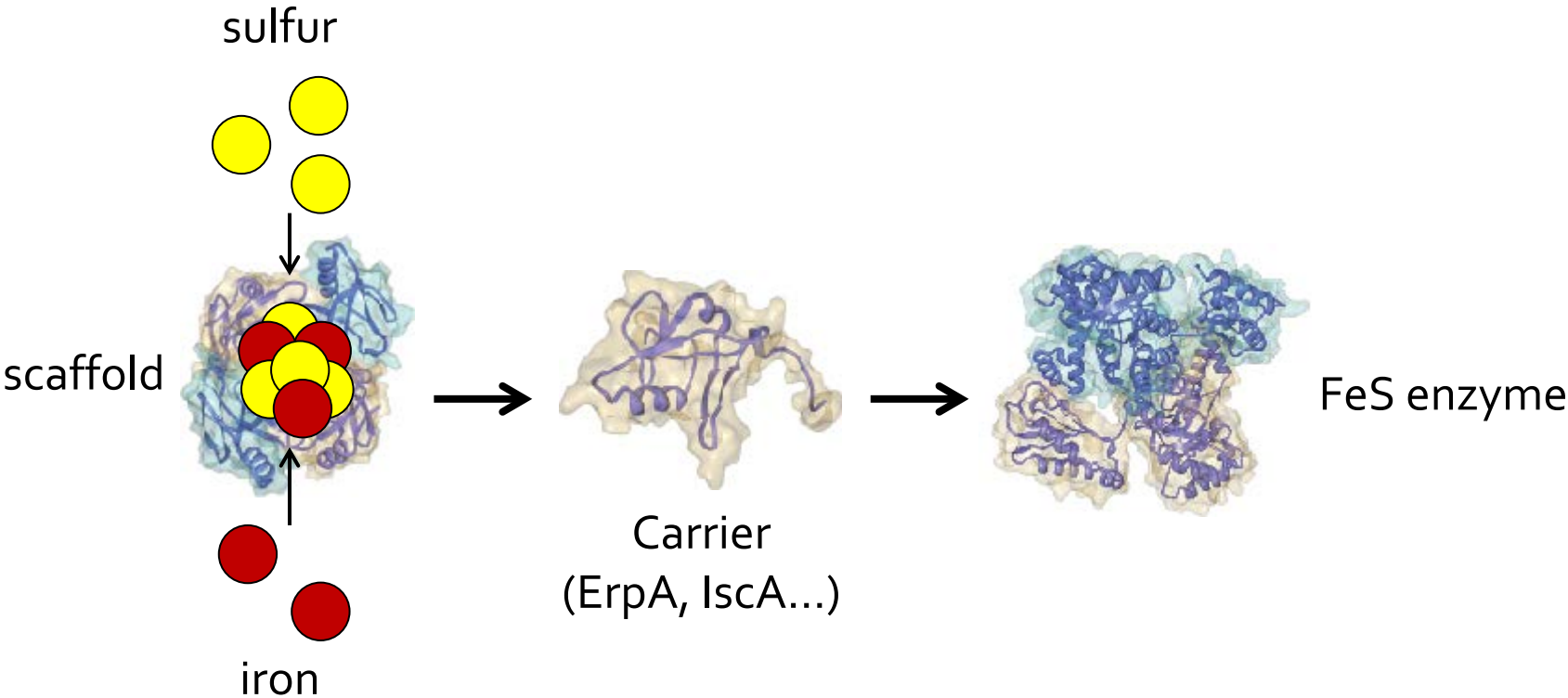


First antimalarial treatments produced  
with semisynthetic artemisinin enter market

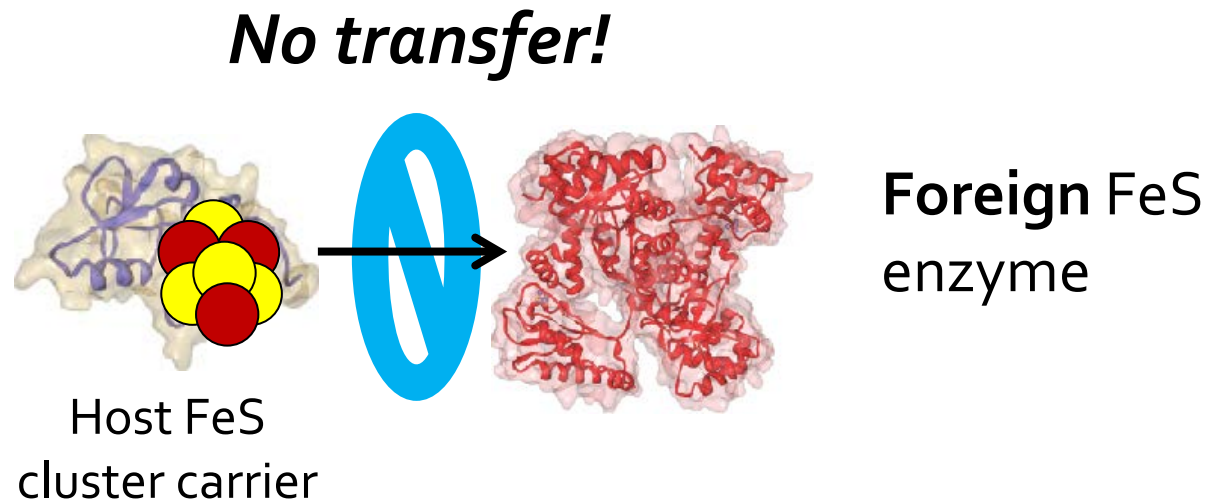


**Enzymes from foreign organisms are not always compatible with engineered hosts!**

# FeS enzymes acquire FeS clusters from specific protein cofactors

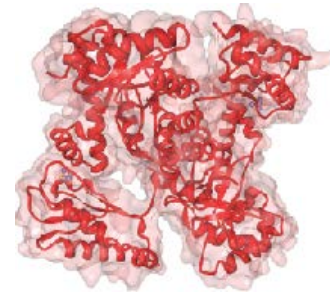


# Foreign FeS proteins often cannot interact with host FeS delivery proteins



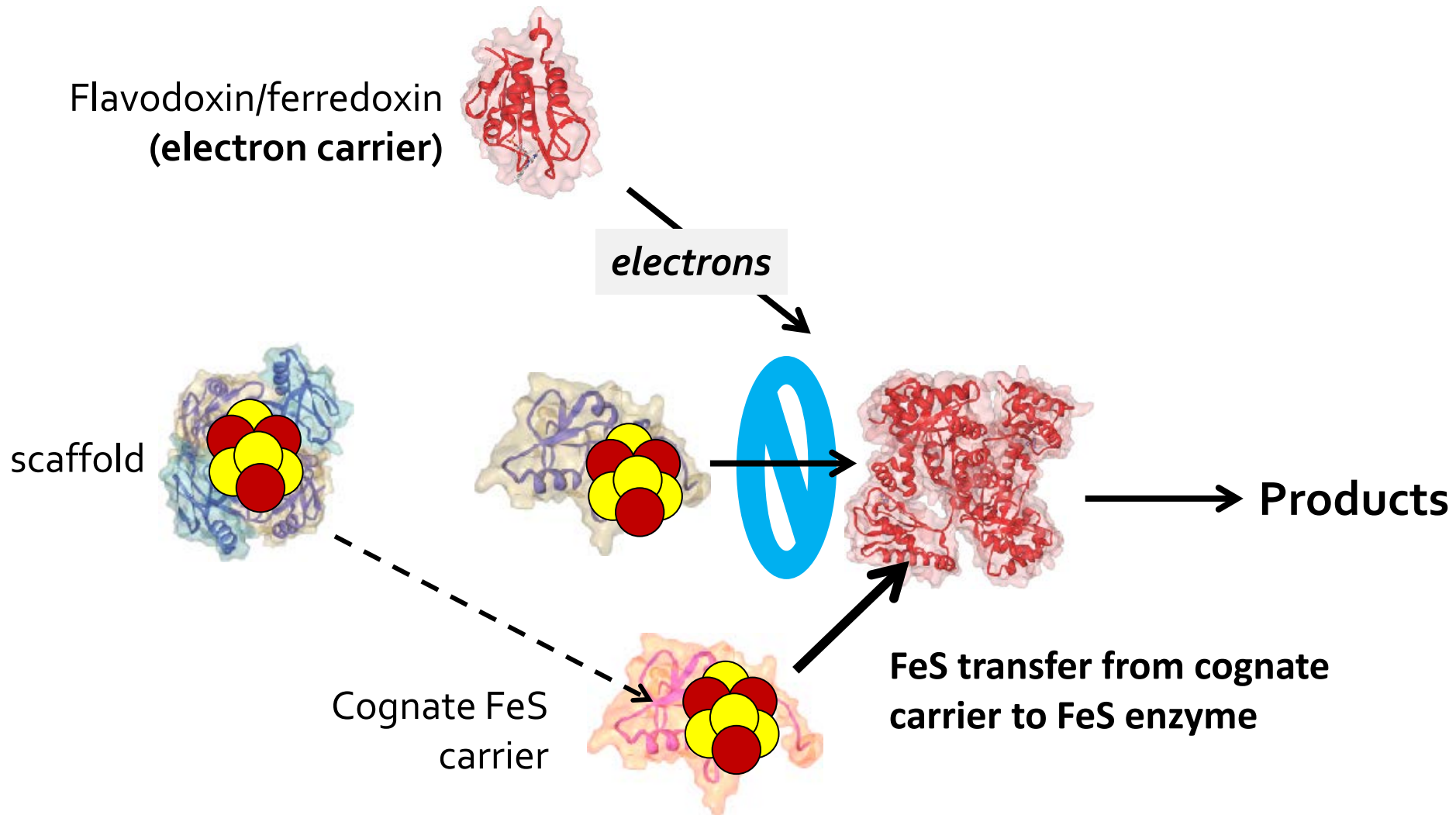
**IRONPLUGNPLAY ambition:**

to reliably activate any FeS enzyme  
from any species within a foreign host

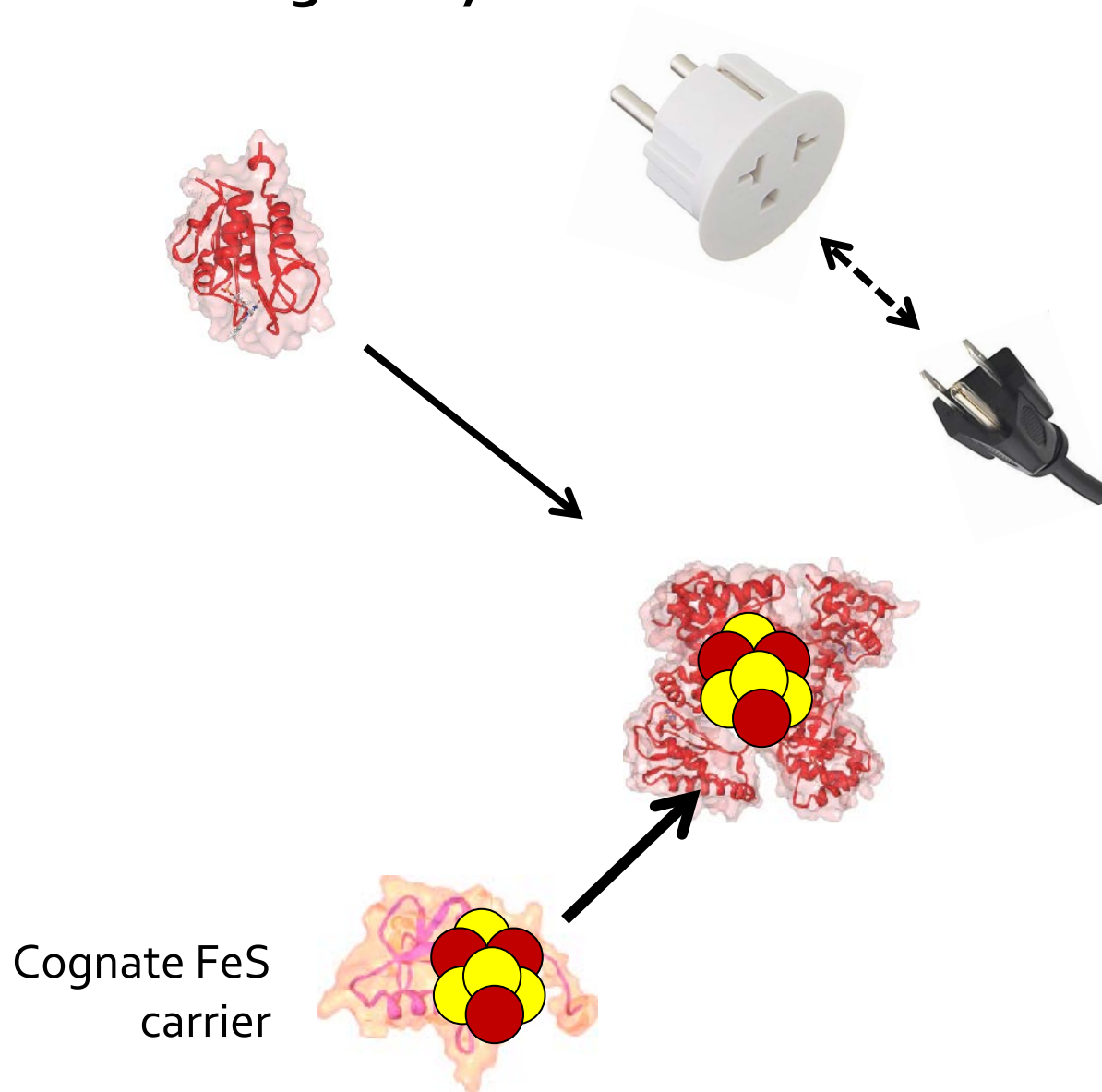


**Foreign FeS  
enzyme**

# Our approach: Co-express FeS cluster carriers and electron carriers that are compatible with the foreign enzyme

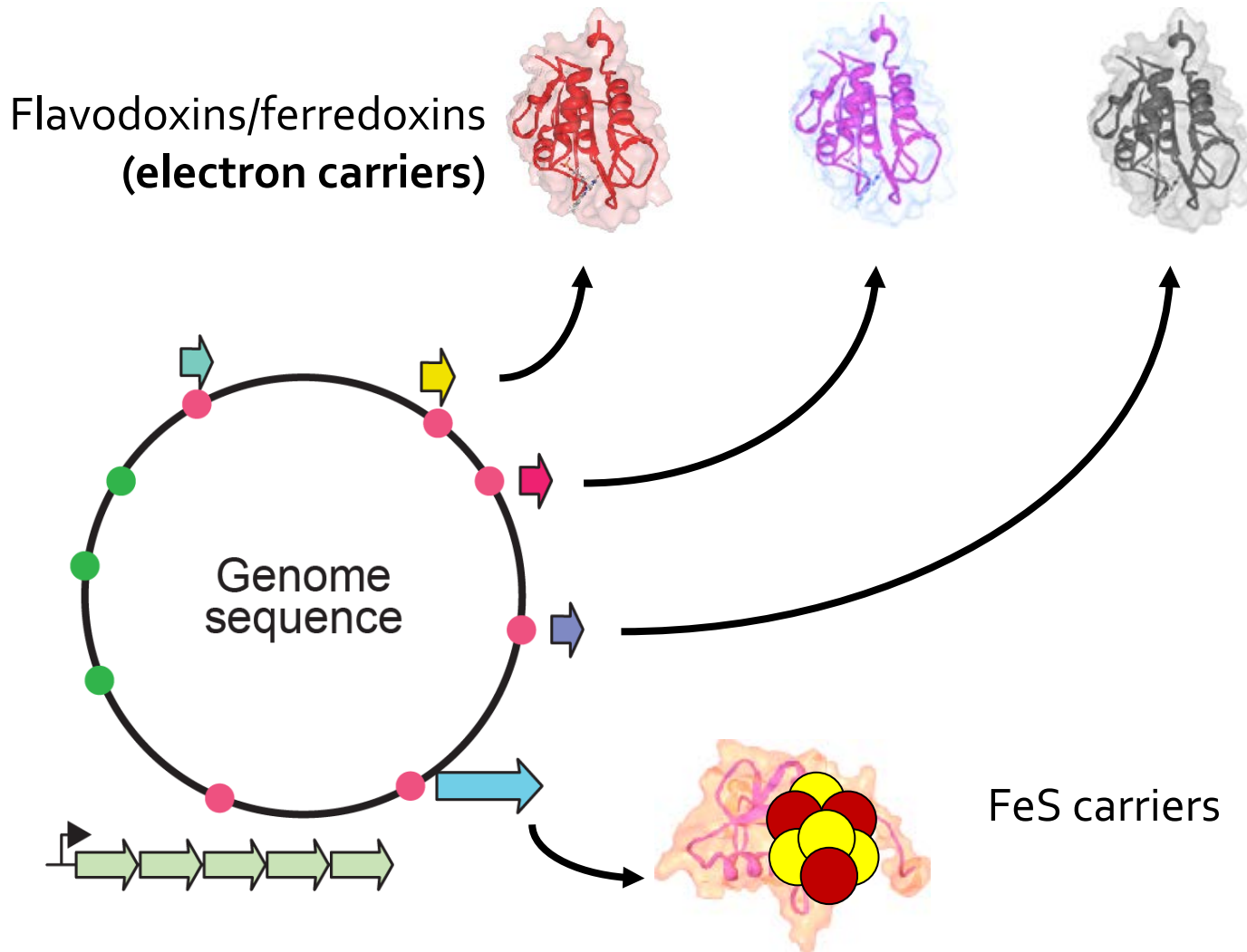


# Carrier proteins effectively act as “plug adapters” for foreign enzymes





# FeS cluster carriers and electron carriers can be identified via genome mining

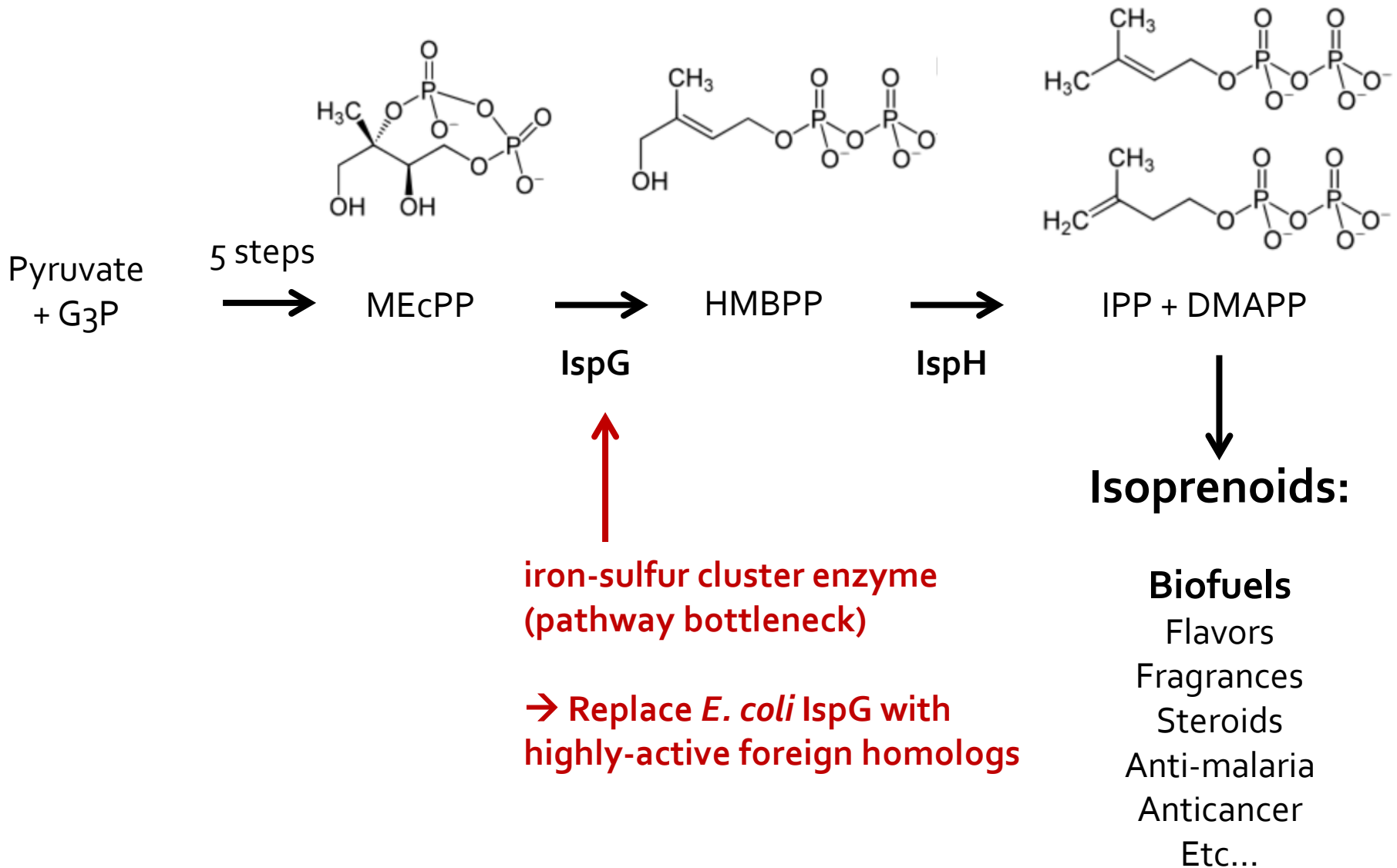


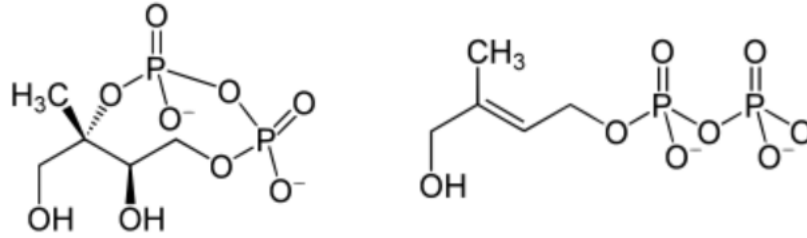
**Can we reliably activate FeS enzymes with carrier proteins?**

**A test case: the bacterial isoprenoid biosynthesis pathway**

# Can we reliably reactivate FeS enzymes?

## *Test with an isoprenoids pathway*





MEcPP



HMBPP

IspG



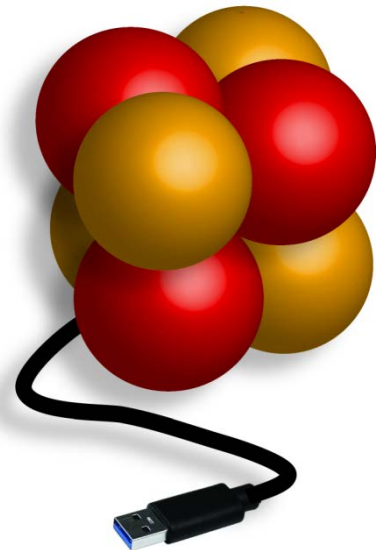
*E. coli* homolog requires:

- FeS cluster carrier: ErpA

- Electron carrier: flavodoxin FldA

## The IRONPLUGNPLAY Project Plan:

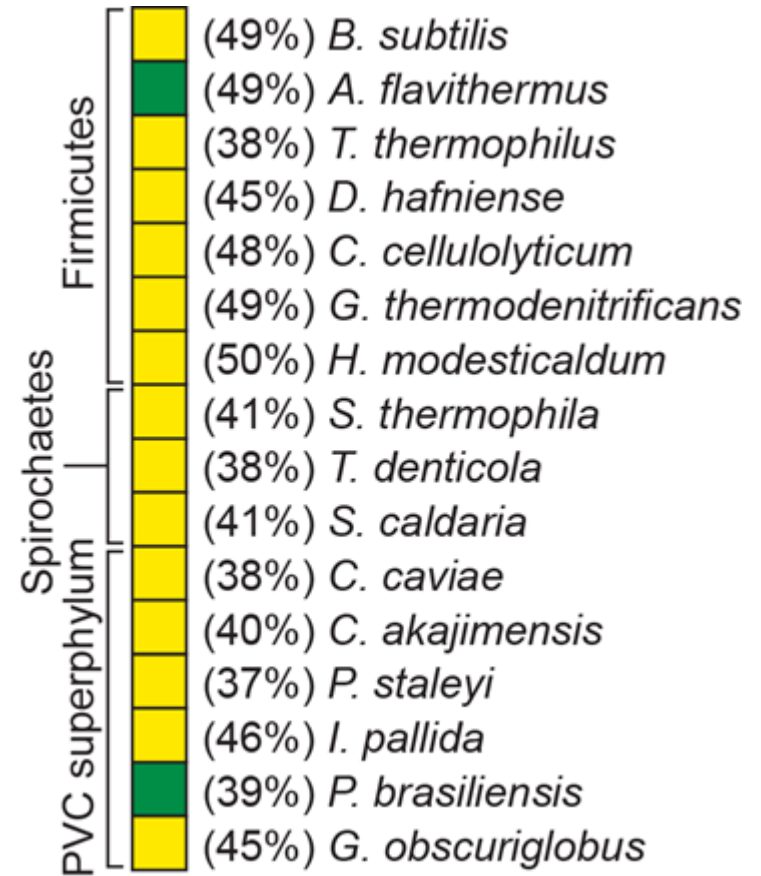
*How to systematically activate FeS enzymes in foreign hosts*



# The IRONPLUGNPLAY Project Plan

## Work Package 1:

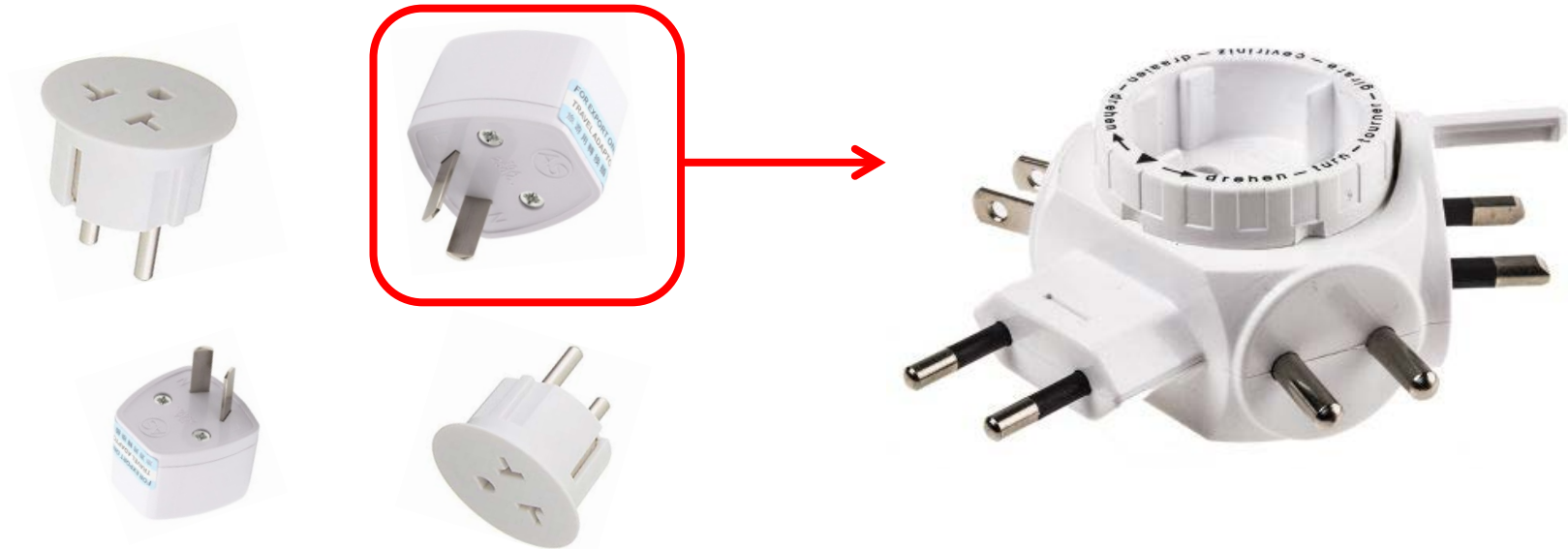
- Identify and test FeS and electron carriers that activate IspG, other FeS enzymes



# The IRONPLUGNPLAY Project Plan

## Work Package 2:

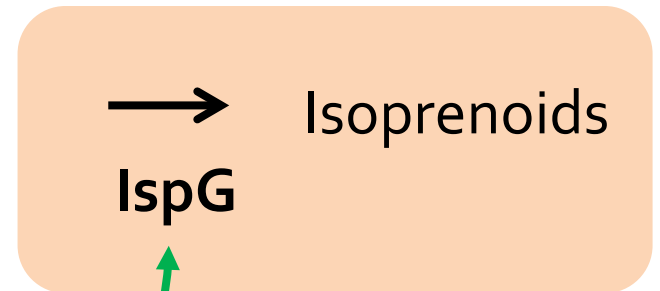
- Use directed evolution to obtain a “universal” FeS carrier



# The IRONPLUGNPLAY Project Plan

## Work Packages 3 & 4:

- Use FeS and electron carriers to improve production of biofuels and fragrances

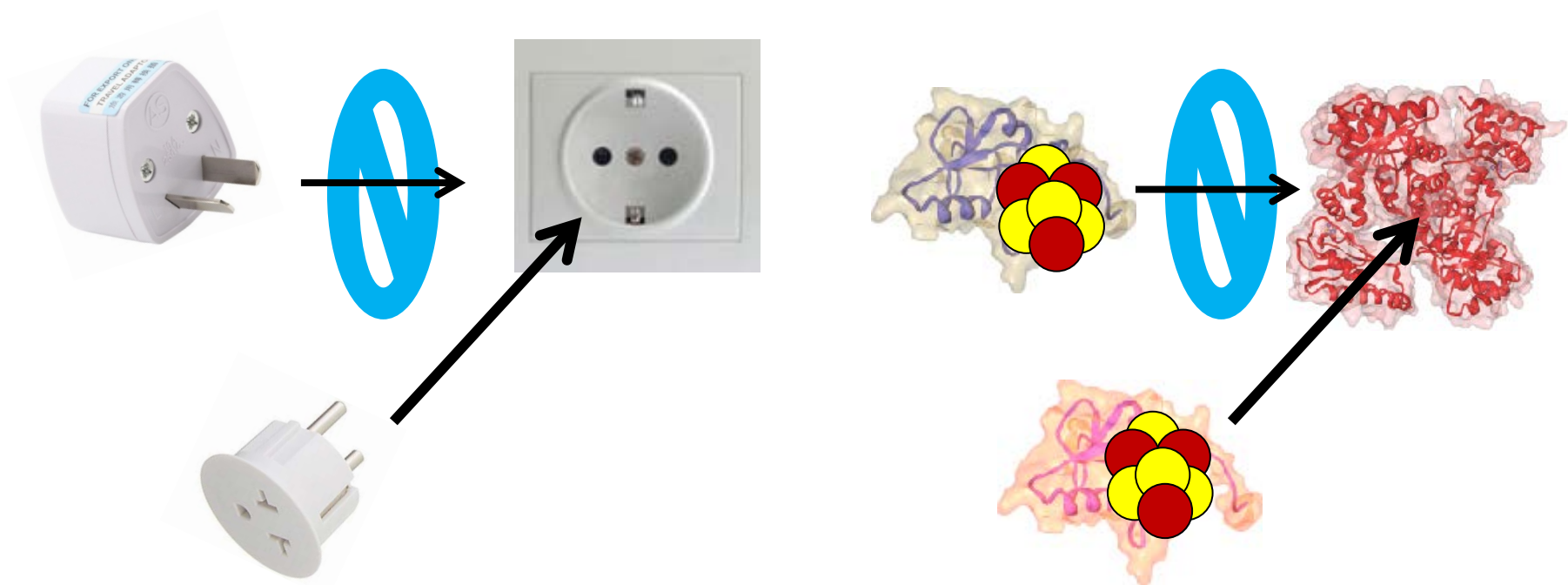




# The IRONPLUGNPLAY Project Plan

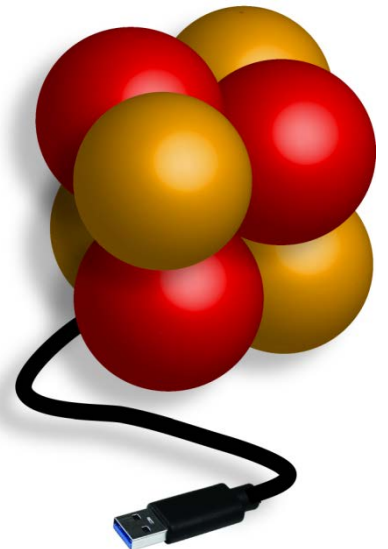
## Work Package 5:

- Understand the determinants of successful vs unsuccessful transfers of FeS clusters



# What will IRONPLUGNPLAY achieve?

- 🍎 **A toolset** for activating FeS enzymes in foreign hosts
- 🍎 **High-yielding isoprenoid pathways** for fuels and fragrances
- 🍎 **An understanding** of what determines successful and unsuccessful FeS cluster transfer



# IRONPLUGNPLAY: Who we are



Miguel Alcalde  
CSIC Madrid



Frederic Barras  
Pasteur Institute

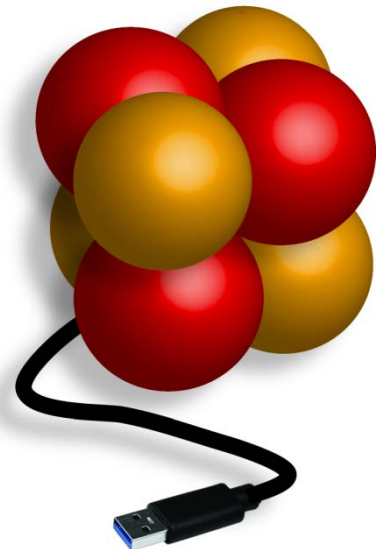


Sandrine  
Ollagnier-de Choudens  
CNRS Grenoble



Geleen, The Netherlands

Coordinator: **Greg Bokinsky**, TU Delft (NL)



# Supporting IRONPLUGNPLAY



## ERA-Net Cofund

National agencies:

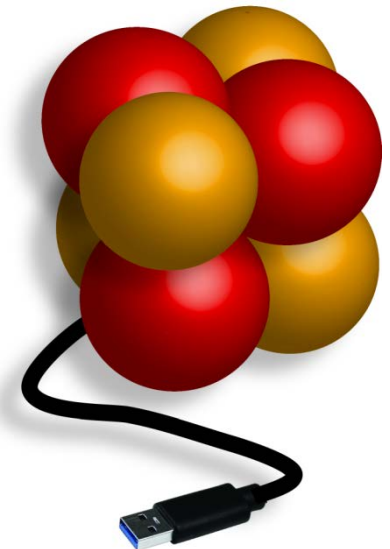
NWO (NL)

ANR (FR)

MINECO (ES)

**Budget: €850,000**

Codon-optimized genes (>500,000 basepairs) provided by the **Joint Genome Institute (USA DOE)**



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