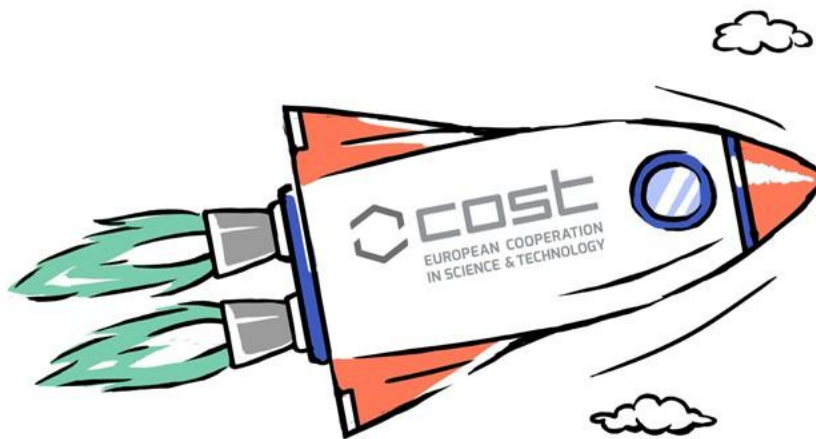




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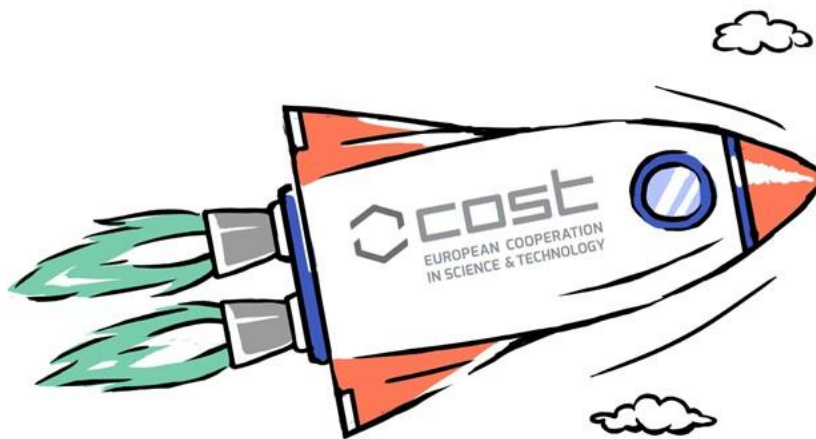
WG2. FeS Clusters in Viral Replication

Deliverables

Deliverable 7. Report on viral/pathogen FeS proteins and their mutants and protocols for their purifications.

Deliverable 8. Report on the interaction of viral/pathogen FeS proteins with components of FeS biogenesis machinery.

Period: Nov 2022 – Nov 2026



Deliverable 7-8

Executive Summary

Working Group 2 (WP2) has achieved substantial progress in elucidating the fundamental role of iron-sulfur (Fe-S) cluster biology in pathogen virulence, host-pathogen interactions, and antiviral defense mechanisms. The outputs from this reporting period demonstrate a comprehensive approach spanning from basic mechanistic enzymology to translational therapeutic development. Key achievements include: (1) a comprehensive framework for understanding the SUF Fe-S biogenesis system as a vulnerability in mycobacterial pathogens; (2) the rational engineering of self-sufficient antiviral enzymes based on radical-SAM Fe-S chemistry; (3) the advancement of mitochondria-targeted iron chelation as a broad-spectrum antiparasitic strategy; and (4) a synthesis of knowledge linking iron homeostasis to immunometabolism in the context of infection. These findings establish Fe-S cluster pathways as both critical determinants of pathogen fitness and promising targets for next-generation antimicrobial and antiviral interventions.

1. Fe-S Clusters as Vulnerabilities in Bacterial Pathogens

1.1. The SUF System as a Therapeutic Target in Mycobacteria (Output 1)

The WP2 has produced a foundational review and analysis of Fe-S cluster biogenesis in mycobacterial species, with particular emphasis on *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis.

- **Finding:** Mycobacteria rely exclusively on the **SUF (sulfur utilization factor)** machinery for Fe-S cluster biogenesis, unlike many other bacteria that possess redundant ISC or NIF systems. This exclusivity renders the SUF pathway a critical vulnerability.
- **Mechanistic Insights:** The review synthesized current knowledge on sulfur mobilization (via SUF-S), iron sourcing, and scaffold assembly (SUF-B/C/D) in

mycobacteria, proposing a global model for [Fe-S] cluster assembly specific to these organisms.

- **Pathogen Relevance:** The work catalogued essential Fe-S proteins in Mtb linked to **virulence, persistence in hypoxic granulomas, central metabolism, and antibiotic resistance** (e.g., electron transport chain components and DNA repair enzymes).
 - **Action Impact:** By identifying specific nodes within the SUF pathway and downstream Fe-S-dependent enzymes, this output provides a roadmap for the development of **novel antimycobacterial agents**. The identification of emerging inhibitors targeting Fe-S-dependent pathways validates this strategy as a viable avenue to combat drug-resistant tuberculosis.
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2. Fe-S Chemistry in Antiviral Host Defense

2.1. Engineering Self-Sufficient Radical-SAM Antiviral Enzymes (Output 2)

A major unresolved question in innate immunity has been how the interferon-stimulated, ER-localized radical-SAM enzyme **Viperin (RSAD2)** acquires electrons for its antiviral nucleotide dehydratase activity. WP2 researchers have solved this mechanistic puzzle and translated the finding into a biotechnological breakthrough.

- **Finding:** The study identified **cytochrome b5 reductase 3 (CYB5R3)** as the physiological electron donor for Viperin in human cells. Electron transfer was shown to be strictly dependent on co-localization at the endoplasmic reticulum membrane.
 - **Structural and Mechanistic Insight:** The researchers obtained structural insights into the RSAD2:CYB5R3 complex and identified specific amino acid residues required for electron transfer, resolving a long-standing question in radical-SAM enzymology regarding ER localization.
 - **Biotechnological Application:** Leveraging this knowledge, the team engineered **iCAREs (immune-silent, catalytically self-sufficient antiviral restriction factor enzymes)**. These autonomous enzymes generate antiviral nucleotide analogues (ddhCTP) to chain-terminate viral RNA polymerases **without requiring host redox partners**.
 - **Action Impact:** This work establishes a general strategy to engineer self-sufficient radical-SAM enzymes. The iCARE platform avoids off-target interference with host redox pathways (e.g., CYB5R3-dependent fatty acid desaturation), offering a safer and more controllable foundation for future antiviral therapeutics targeting RNA viruses.
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3. Targeting Iron and Fe-S Metabolism in Eukaryotic Pathogens

3.1. Mitochondria-Targeted Iron Chelation Against Parasites and Fungi (Output 4)

Host iron sequestration is a critical innate immune defense, yet unicellular parasites have evolved sophisticated iron-scavenging strategies. WP2 has advanced a novel therapeutic approach to overcome this barrier.

- **Finding:** The development of **mitoDFO**, a mitochondrially targeted derivative of the iron chelator deferoxamine (DFO), demonstrates potent in vitro efficacy against a broad range of unicellular pathogens.
- **Parasitic Activity:** Intracellular *Leishmania* infection was cleared by mitoDFO, indicating its capacity to access and disrupt the pathogen within host cells. Experiments with *Trypanosoma brucei* revealed that the mechanism extends beyond simple iron starvation.
- **Mechanistic Insight:** mitoDFO not only disrupts parasite iron homeostasis but also **alters the physicochemical properties of the inner mitochondrial membrane**, leading to loss of mitochondrial function. This dual mechanism—targeting both Fe-S cluster biogenesis and membrane integrity—is highly promising.
- **Broad-Spectrum Validation:** Investigating virulence factors in pathogenic yeasts confirmed mitoDFO as a viable candidate against a wide spectrum of microbe-associated diseases, positioning mitochondrial iron metabolism as a universal vulnerability in eukaryotic pathogens.

3.2. Iron Homeostasis and Immunometabolism in Host Defense (Output 3)

Complementing the pathogen-focused studies, WP2 contributed to a major review examining the bidirectional relationship between iron metabolism and host immune function during physiological stress and infection.

- **Finding:** The review highlights how immune cells must balance iron availability: ensuring sufficient supply for anabolic metabolism and immune effector functions (e.g., cytokine production, proliferation) while simultaneously **restricting iron access to invading pathogens** (nutritional immunity).
- **Metabolic Integration:** The work maps the critical dependence of immunometabolism on iron homeostasis, covering the citric acid cycle (aconitase), oxidative phosphorylation (respiratory complexes), glycolysis, the pentose phosphate pathway, and fatty acid oxidation.
- **Action Impact:** This synthesis provides a conceptual framework for interpreting WP2's experimental findings. Disruptions in host iron handling contribute directly to susceptibility and immunopathology. The review underscores that therapeutic strategies (such as chelation or Fe-S pathway inhibition) must consider the delicate balance between host immune function and direct antimicrobial effects.

4. Synthesis and Future Directions for WP2

The outputs from this reporting period reveal a unifying theme: **Fe-S cluster biology represents a conserved and exploitable vulnerability across diverse pathogen classes.**

Pathogen Class	Key Fe-S Vulnerability	WP2 Contribution
Bacteria (Mycobacteria)	Exclusive dependence on SUF system	Roadmap for SUF-targeted inhibitors
Viruses (RNA viruses)	Viperin-dependent nucleotide synthesis	Engineering autonomous iCARE enzymes
Parasites (Leishmania, Trypanosoma)	Mitochondrial Fe-S metabolism and membrane integrity	Development of mitoDFO
Fungi (Pathogenic yeasts)	Mitochondrial iron utilization	Validation of mitoDFO broad-spectrum activity

Next Steps for WP2:

1. **Mycobacterial Validation:** We will prioritize the experimental validation of predicted essential Fe-S enzymes in *M. tuberculosis* under hypoxic (granuloma-mimicking) conditions to identify the most promising drug targets within the SUF pathway.
2. **iCARE Translation:** Building on the successful proof-of-concept, WP2 will initiate a Short-Term Scientific Mission (STSM) to test iCARE efficacy in primary human immune cells infected with RNA viruses of pandemic concern.
3. **mitoDFO Mechanistic Depth:** We will combine mitoDFO treatment with transcriptomic and proteomic analyses in *Leishmania* to fully map the cellular response to mitochondrial iron depletion and membrane disruption.
4. **Cross-Working Group Integration:** WP2 will integrate the mitoNEET hypoxia-sensing model (developed in WP1/WG3) to investigate whether host mitochondrial O₂ sensing via Fe-S clusters influences the outcome of intracellular pathogen infections.

Disclaimer: This report is a synthesis document prepared for COST Action Working Group 2 based on the provided abstracts. Specific grant numbers and author lists are omitted for brevity but are available in the original publications.

