

COST Action CA21115

Report date: October 2026

WG1. FeS Clusters and the Immune System

Deliverables

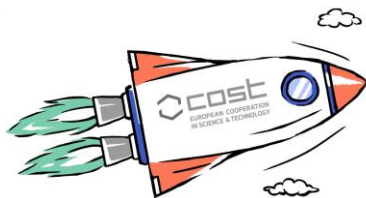
Deliverable 2. Report on Cell-line models of knockout or mutants of components of FeS biogenesis machinery.

Deliverable 3. Report on data regarding the activation of FeS biogenesis in response to infectious agents.

Deliverable 4. Report on metabolomics analysis of the role of FeS biogenesis in cellular chemistry.

Deliverable 5. Publication. Scientific research and review articles describing the methods and findings.

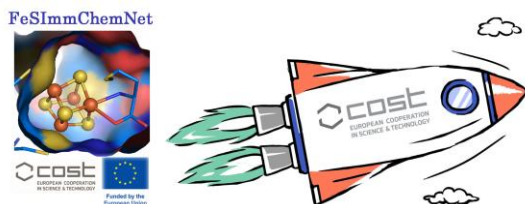
Period: Nov 2022 – Nov 2026



Deliverable 2.

Report cell lines

Conditional mutants of Fe-S biogenesis components	Organism	Pubmed ID
HCF101 (CIA transfer protein)	<i>Toxoplasma gondii</i>	PMID: 39913537
NAR1 (CIA shuttle protein)	<i>Toxoplasma gondii</i>	PMID: 39913537
SUFC (SUF scaffold component)	<i>Toxoplasma gondii</i>	PMID: 35810787
NFS2 (SUF sulfur-providing enzyme)	<i>Toxoplasma gondii</i>	PMID: 34793583
ISU1 (ISC scaffold component)	<i>Toxoplasma gondii</i>	PMID: 34793583
Mutant of nitrogenase FeS biogenesis	<i>Azotobacter vinelandii</i>	
Component of FeS cluster biogenesis for nitrogenase	<i>Escherichia coli</i> , <i>Klebsiella oxytoca</i> , <i>Saccharomyces cerevisiae</i> , <i>Oriza sativa</i> , <i>Medicago truncatula</i> , <i>Nicotiana benthamiana</i> , and <i>Arabidopsis thaliana</i>	
RSAD2KO	<i>Macrophages and A549</i>	



Deliverable 3-4.

Executive Summary

This reporting period has yielded significant advancements in understanding the intersection of iron-sulfur (Fe-S) protein biology, cellular iron homeostasis, and immune function. The Action has successfully fostered interdisciplinary collaboration resulting in publications that bridge analytical chemistry, immunology, and structural biology. Key findings include the development of a novel single-cell methodology to quantify iron in immune cells, the elucidation of the mechanistic function of the mitoNEET Fe-S protein in gasotransmitter signaling, and the engineering of a self-sufficient antiviral enzyme based on radical-SAM Fe-S cluster chemistry. These outputs collectively validate the Action's objective to dissect the role of Fe-S proteins in metabolism and immune regulation, providing both fundamental mechanistic insights and novel biotechnological tools.

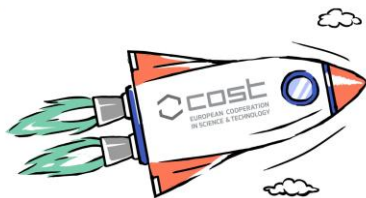
1. Key Findings: Fe-S Proteins and the Immune System

The Action has made decisive progress in connecting Fe-S cluster chemistry to immune cell function, moving beyond correlation to establish mechanistic pathways.

1.1. Unraveling the "Iron Paradox" in T Cells (Output 1)

A persistent challenge in immunometabolism has been the accurate measurement of labile iron pools in small, non-adherent cells like lymphocytes. The Action supported the application of **Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)** to primary human T cells.

- **Finding:** The study established a reproducible quantification of iron at the femtogram level in single cells. It revealed that resting T cells maintain strict, donor-independent iron homeostasis. However, upon activation, T cells exhibit heterogeneous iron uptake patterns, suggesting that distinct differentiatonal pathways (e.g., effector vs. memory) impose specialized metabolic demands.
- **Action Impact:** This work provides the first rigorous evidence that the "iron deficiency/overload" paradigm in T cell dysfunction is not a bulk phenomenon but a cell-specific event, directly linking Fe-S cluster biogenesis demands to T cell fate decisions.



1.2. The mitoNEET [2Fe-2S] Cluster as a Hypoxia and NO Sensor (Outputs 4 & 5)

The mitochondrial outer membrane protein mitoNEET has long been associated with metabolism and diabetes drugs (pioglitazone), but its molecular function was unresolved. The Action's collaborative approach (combining computational modeling with biophysics) has resolved this long-standing question.

- **Finding:** Researchers identified an **explicit nitric oxide (NO) access site** to the mitoNEET [2Fe-2S] cluster. Critically, the study demonstrated that molecular oxygen (O_2) and the drug pioglitazone physically block NO access to the cluster.
- **Mechanistic Insight:** The data support a model where the mitoNEET cluster acts as a **gasotransmitter sensor**. Under normoxia, O_2 binds/protects the cluster, stabilizing the protein. Under hypoxia, O_2 dissociates, allowing NO to attack the cluster and initiate downstream mitochondrial signaling (affecting fission/fusion and respiration).
- **Action Impact:** This is a paradigm shift in understanding eukaryotic Fe-S proteins. It establishes that [Fe-S] clusters are not merely structural or electron transfer cofactors but can serve as dynamic sensors for gaseous signaling molecules, with direct implications for inflammatory responses and age-related immune decline.

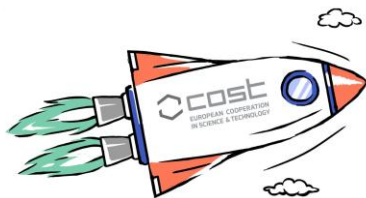
1.3. Engineering Immune-Silent Antiviral Enzymes (Output 3)

Translating the Action's fundamental chemistry knowledge into applied science, researchers addressed the mechanism of Viperin (RSAD2), a radical-SAM enzyme essential for antiviral immunity.

- **Finding:** The study identified **CYB5R3** as the endogenous electron donor for Viperin in the endoplasmic reticulum. By solving the structural requirements for this partnership, the team engineered **iCAREs (immune-silent, catalytically self-sufficient antiviral restriction factor enzymes)**.
- **Action Impact:** This work proves that understanding Fe-S cluster electron transfer chains allows for the creation of "autonomous" enzymes that do not compete with host redox partners (like CYB5R3). This has significant potential for future antiviral therapeutics and biotechnological applications, fulfilling the Action's goal of exploiting Fe-S chemistry for societal benefit.

2. Key Findings: Fe-S Proteins and Metabolism

The Action has strengthened the conceptual framework linking Fe-S cluster biogenesis to core metabolic pathways.



2.1. Bidirectional Interplay of Iron and Immunometabolism (Output 2)

A comprehensive review produced by the Action synthesized the intricate relationship between iron homeostasis and metabolic pathways in the context of lung health and disease.

- **Finding:** The review highlights the critical dependence of effector immune functions on efficient iron handling. It mapped the specific points where iron (and by extension, Fe-S clusters) interfaces with the **Citric Acid Cycle (aconitase), Oxidative Phosphorylation (Complex I-III), and glycolysis**.
- **Action Impact:** The publication emphasized that disruptions in iron handling are not merely a side effect of metabolic stress but a driver of immunopathology. This positions Fe-S cluster biogenesis machinery as a potential therapeutic target for metabolic inflammatory diseases (e.g., chronic lung disease).

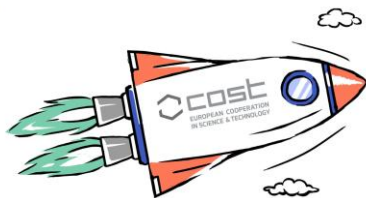
3. Conclusion and Future Directions

The published outputs from this period demonstrate the Action's success in fostering a "cluster-to-clinic" understanding of Fe-S proteins. The key conclusions are:

1. **Fe-S Clusters are Signal Transducers:** The discovery of the mitoNEET O_2/NO sensing mechanism proves that Fe-S clusters in the immune system act as active signaling modules, not passive cofactors.
2. **Single-cell Heterogeneity is Critical:** The LA-ICP-MS study shows that immune activation creates diverse iron metabolic states; future therapeutic interventions targeting iron metabolism must account for this heterogeneity.
3. **Mechanistic Translation is Viable:** The successful creation of iCAREs validates that deep mechanistic knowledge of Fe-S electron transfer can be directly translated into biotechnological tools to combat viral threats.

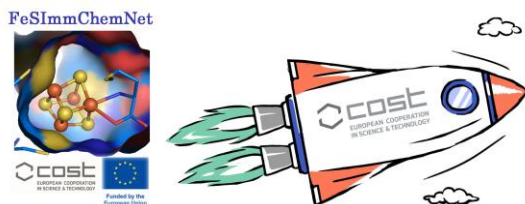
Next Steps:

- **Working Group Integration:** We will integrate the mitoNEET hypoxia-sensing model with the T cell activation data to test whether mitochondrial O_2 sensing via Fe-S clusters regulates T cell differentiation (e.g., Th17 vs. Treg).
- **Therapeutic Development:** Building on the iCARE proof-of-concept, we will explore modifications to enhance the stability of these enzymes for potential use in primary immune cells.



- **Network Expansion:** We will initiate a Short-Term Scientific Mission (STSM) focused on applying LA-ICP-MS to cells with mutated Fe-S assembly factors to validate the direct link between cluster biogenesis and cellular iron uptake.

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



Deliverable 5.

Publications

1. Dark proteome ensemble superposition gating calibrates biosynthesis of antiviral cocktail, BioRxiv DOI: <https://doi.org/10.64898/2026.09.10.750538>
2. Single-cell assessment of iron content in primary human T cells using laser ablation inductively coupled plasma mass spectrometry, Cell Report Methods (2026) Volume 6, Issue 4101343. DOI: [10.1016/j.crmeth.2026.101343](https://doi.org/10.1016/j.crmeth.2026.101343)
3. Iron and immunometabolism: Perspectives from the lung, Current Opinion in Endocrine and Metabolic Research (2026) Volume 44, 100613. DOI: <https://doi.org/10.1016/j.coemr.2026.100613>
4. Discovery of an Antiviral Electron Transfer Process to Create Catalytically Self-Sufficient Viral Restriction Factors, Angewandte Chemie (2026), e5423408. DOI: <https://doi.org/10.1002/anie.5423408>
5. Emerging role of mitoNEET as mitochondrial sensor of hypoxia. Journal of Biological Inorganic Chemistry (2026) Volume 31, pages 119–130. DOI: <https://doi.org/10.1007/s00775-026-02139-y>
6. The [2Fe-2S] cluster of mitochondrial outer membrane protein mitoNEET has an O₂-regulated nitric oxide access tunnel, FEBS Letters (2025) Volume 599, Issue 7, Pages 952–970. DOI: <https://doi.org/10.1002/1873-3468.15097>
7. Ancient complexes of iron and sulfur modulate oncogenes and oncometabolism, Current Opinion in Chemical Biology (2023) Volume 76, 102338. DOI: <https://doi.org/10.1016/j.cbpa.2023.102338>
8. Radical-SAM dependent nucleotide dehydratase (SAND), rectification of the names of an ancient iron-sulfur enzyme using NC-IUBMB recommendations, Front. Mol. Biosci. (2022), Volume 9, DOI: <https://doi.org/10.3389/fmolb.2022.1032220>