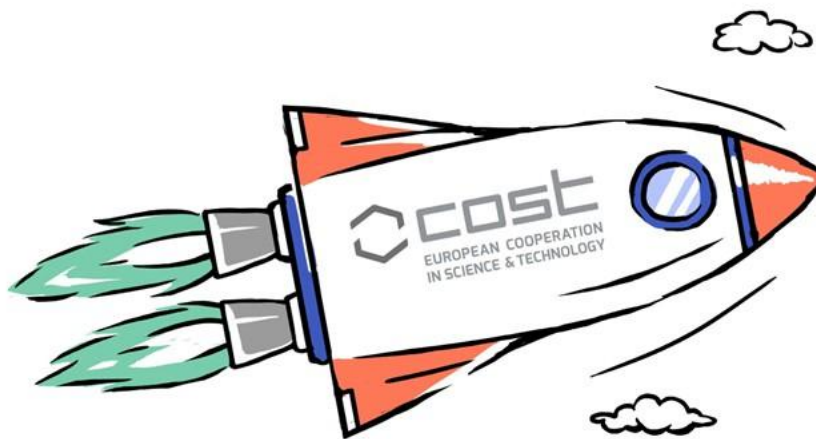




COST Action CA21115

Report date: October 2026

WG5. FeS clusters and proteins as drug target

Deliverables

Deliverable 19. Report on a set of potential small molecule candidates for developing therapeutics to target FeS proteins.

Deliverable 20. Papers on targeting FeS proteins for treating infectious diseases and other human diseases like cancer and neurodegeneration.

Deliverable 21. Papers on targeting specific cellular proteins to boost the immune response against a wide range of viruses.

Period: Nov 2022 – Nov 2026

Deliverable 19

Executive Summary

Working Group 5 (WP5) has made substantial progress in translating fundamental knowledge of iron-sulfur (Fe-S) cluster biology and metalloprotein chemistry into therapeutic applications and biotechnological innovations. The outputs from this reporting period demonstrate a comprehensive pipeline spanning from target identification and validation to drug development and protein engineering. Key achievements include: (1) establishment of the mycobacterial SUF system as a validated drug target with identified inhibitor opportunities; (2) development of mitochondria-targeted iron chelators (mitoDFO) with broad-spectrum antiparasitic and antifungal activity; (3) engineering of immune-silent, self-sufficient antiviral enzymes (iCAREs) based on radical-SAM Fe-S chemistry; (4) structural validation of broad-spectrum antiviral PROTACs targeting viral proteases; (5) development of a versatile ferritin nanocage engineering platform for drug delivery and vaccine development; (6) creation of biocompatible molecularly imprinted polymers for metalloenzyme immobilization; and (7) characterization of a novel copper-biliverdin complex with potential anticancer applications. These outputs collectively establish WP5 as a driving force in translating Fe-S and metalloprotein research into tangible therapeutic and biotechnological applications.

1. FeS Proteins as Drug Targets in Infectious Diseases

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

Tuberculosis remains a global health crisis, exacerbated by the emergence of multidrug-resistant strains. WP5 has established a comprehensive framework for targeting Fe-S cluster biogenesis in *Mycobacterium tuberculosis* (Mtb).

- **Target Identification:** Mycobacteria rely **exclusively on the SUF (sulfur utilization factor)** machinery for Fe-S cluster biogenesis, unlike other bacteria possessing redundant ISC or NIF systems. This exclusivity renders the SUF pathway a critical vulnerability.
- **Druggability Assessment:** The review synthesized current knowledge of:
 - Cellular sources of iron and sulfur in mycobacteria.
 - A global model for [Fe-S] cluster assembly via the SUF system.
 - Essential Fe-S proteins in Mtb linked to **virulence, persistence in hypoxic granulomas, metabolism, and antibiotic resistance**.
- **Therapeutic Opportunities:** The review highlights **emerging inhibitors targeting Fe-S-dependent pathways** and their potential as antimycobacterial agents. Specific

nodes within the SUF pathway (SufS, SufU, SufB/C/D) and downstream Fe-S enzymes represent druggable targets.

- **Action Impact:** This output provides a strategic roadmap for developing novel antitubercular agents that exploit the unique Fe-S biogenesis requirements of mycobacteria, with potential to overcome existing drug resistance mechanisms.

1.2. Mitochondria-Targeted Iron Chelation Against Eukaryotic Pathogens (Output 7)

Host iron sequestration represents a critical innate immune defense, yet unicellular parasites have evolved sophisticated iron-scavenging strategies. WP5 has advanced a novel therapeutic approach to overcome this barrier.

- **Therapeutic Strategy:** The development of **mitoDFO**, a mitochondrially targeted derivative of the FDA-approved iron chelator deferoxamine (DFO), represents a precision approach to targeting pathogen iron metabolism.
- **Broad-Spectrum Activity:**
 - **Intracellular *Leishmania* infection** was cleared by mitoDFO, demonstrating efficacy against intracellular parasites.
 - **Pathogenic yeasts** were also susceptible, confirming broad-spectrum activity against diverse microbe-associated diseases.
- **Dual Mechanism of Action:** Experiments with *Trypanosoma brucei* revealed that mitoDFO:
 1. Disrupts parasite iron homeostasis.
 2. **Alters the physicochemical properties of the inner mitochondrial membrane**, resulting in loss of mitochondrial function.
- **Action Impact:** This dual mechanism—targeting both Fe-S cluster biogenesis and membrane integrity—represents a significant advantage over conventional chelators. The work establishes mitochondrial iron metabolism as a universal vulnerability in eukaryotic pathogens and positions mitoDFO as a promising clinical candidate.

1.3. Broad-Spectrum Antiviral PROTACs Targeting Viral Proteases (Output 6)

Viral proteases represent ideal drug targets due to their conservation across viral families and absence of homologous enzymes in humans. WP5 has advanced a novel protein degradation strategy with broad-spectrum antiviral potential.

- **Therapeutic Strategy:** A **GC-376-based PROTAC** (PROteolysis TArgeting Chimera) was developed to target and induce proteasome-mediated degradation of viral 3C/3CL proteases, rather than merely inhibiting their catalytic activity.
- **Structural Validation:** X-ray crystallography of the CVB3 3CPro protease bound to the GC-376 PROTAC precursor was obtained at **1.9 Å resolution**, revealing:
 - Strong overall similarity to SARS-CoV-2 3CLPro binding (RMSD 0.3 Å on C-alpha).

- Notable variation in the orientation of the benzyloxycarbonyl group at the S4 subsite.
 - **NMR Characterization:** Backbone assignments (80% and 97% for bound and unbound states) enabled detailed analysis of the interaction, revealing that the PROTAC binds in a mode very similar to its precursor.
 - **Dynamic Insights:** NMR relaxation data indicated that **substrate binding quenches backbone dynamics** of the substrate-binding site, suggesting a conformational selection mechanism for substrate recognition.
 - **Action Impact:** The demonstration that GC-376 PROTAC fits well within both coronavirus and enterovirus protease binding sites establishes its **potential as a broad-spectrum antiviral agent** with a novel mechanism of action (targeted degradation rather than inhibition).
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2. Engineering FeS Proteins and Metalloproteins for Therapeutic Applications

2.1. Immune-Silent Self-Sufficient Antiviral Enzymes (iCAREs) (Output 5)

A major challenge in developing enzyme-based therapeutics is the requirement for endogenous cofactors and redox partners. WP5 has engineered autonomous enzymes that overcome this limitation.

- **Engineering Strategy:** Based on structural insights into the Viperin-CYB5R3 electron transfer partnership, researchers designed **iCAREs (immune-silent, catalytically self-sufficient antiviral restriction factor enzymes)**.
- **Key Innovation:** iCAREs autonomously generate antiviral nucleotide analogues (ddhCTP) to chain-terminate viral RNA polymerase activity **without requiring recruitment of endogenous redox partners**.
- **Therapeutic Advantages:**
 - **Immune-silent:** iCAREs avoid interference with host biological processes associated with redox partners like CYB5R3 (e.g., fatty acid desaturation by SCD1).
 - **Self-sufficient:** The enzymes function independently of cellular redox machinery, enabling predictable and controllable activity.
- **Action Impact:** This work establishes a **general strategy to engineer self-sufficient radical-SAM enzymes** for biotechnological and therapeutic applications. The iCARE platform provides a foundation for developing autonomous antiviral therapeutics with minimized off-target effects.

2.2. Ferritin Nanocages for Drug Delivery and Vaccine Development (Output 4)

Naturally occurring protein nanocages like ferritin offer unique advantages for biomedical applications due to their biocompatibility and self-assembly properties. WP5 has developed a versatile engineering platform for these structures.

- **Engineering Strategy:** Inspired by the HIV-1 Gag polyprotein precursor, researchers designed **PREcursors of nanoCages (PREC)** by linking two ferritin subunits via a flexible linker containing a protease cleavage site.
- **Versatile Platform:** Protease cleavage generates ferritin-like nanocages termed **PINCs (protease-induced nanocages)**, enabling:
 - **Surface decoration** with proteins (e.g., antigens for vaccine development).
 - **Hydrophilic drug encapsulation** in the interior cavity.
 - **Hydrophobic drug encapsulation** within the protein shell.
- **Enhanced Capacity:** PINC formation allows drug encapsulation **up to fourfold more** than achieved using other methods.
- **Therapeutic Validation:** The PINCs/Drug complex demonstrated stability and **efficient killing of cancer cells**.
- **Action Impact:** This engineering approach provides a versatile platform for:
 - **Drug delivery systems** with high loading capacity.
 - **Mosaic vaccines** displaying multiple antigens.
 - **Multifunctional therapeutics** combining targeting, imaging, and therapeutic modalities.

2.3. Molecularly Imprinted Polymers for Metalloenzyme Immobilization (Output 3)

The exploitation of metalloenzymes in biotechnology often requires immobilization on solid supports while preserving catalytic activity. WP5 has developed a biocompatible platform for this purpose.

- **Engineering Strategy:** A **polyscopoletin-based molecularly imprinted polymer (MIP)** was developed that recognizes recombinant proteins via imprinting of the widely used **Strep-tag II affinity peptide**.
- **Platform Validation:** The Strep-MIP platform was verified with two Strep-tagged enzymes:
 - An **O₂-tolerant [NiFe]-hydrogenase** (a metalloenzyme of industrial relevance).
 - An **alkaline phosphatase** (a widely used diagnostic enzyme).
- **Performance:** The enzymes **preserved their biocatalytic activities after multiple utilization**, confirming the efficiency of Strep-MIP as a general biocompatible platform.
- **Action Impact:** This platform enables:
 - Confinement of recombinant metalloenzymes for biotechnological applications.
 - Development of enzyme-based biosensors and bioelectrodes.

- General applicability to any Strep-tagged protein, facilitating high-throughput enzyme immobilization.
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3. Novel Metalloprotein-Based Therapeutics

3.1. Copper-Biliverdin Complex with Anticancer Potential (Output 2)

The interactions between metal ions and endogenous tetrapyrroles have been underexplored as potential therapeutic agents. WP5 has characterized a novel complex with promising cytotoxic properties.

- **Finding:** The **Cu²⁺-biliverdin complex (BV-Cu)** is stable in water at physiological pH and exhibits **significantly greater cytotoxicity** to human cell lines than equimolar Cu²⁺ and biliverdin individually.
 - **Structural Characterization:**
 - **XANES spectroscopy** revealed that the complex contains **Cu¹⁺** with distorted tetrahedral or square planar geometry.
 - **DFT modeling** corroborated charge and spin density redistribution between Cu²⁺ and the tetrapyrrole ring during complex formation.
 - **Mechanistic Insights:**
 - The complex is nominally composed of **Cu¹⁺ and BV radical cation**, resulting from a redox reaction during complex formation.
 - **Micro-XRF mapping** revealed that BV-Cu-treated cells exhibit **almost even distribution of Cu throughout the cytosol**, in contrast to the physiological compartmentalization observed in Cu²⁺-treated cells.
 - **Therapeutic Hypothesis:** The cytotoxicity of BV-Cu likely results from **interference with regular Cu transport and storage routes**, suggesting a novel mechanism for anticancer activity.
 - **Action Impact:** This work identifies a novel class of metallotetrapyrrole complexes with potential anticancer applications and provides mechanistic insights that could guide the design of improved therapeutic agents.
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4. Synthesis and Future Directions for WP5

The outputs from this reporting period demonstrate WP5's comprehensive approach to translating Fe-S and metalloprotein research into therapeutic and biotechnological applications.

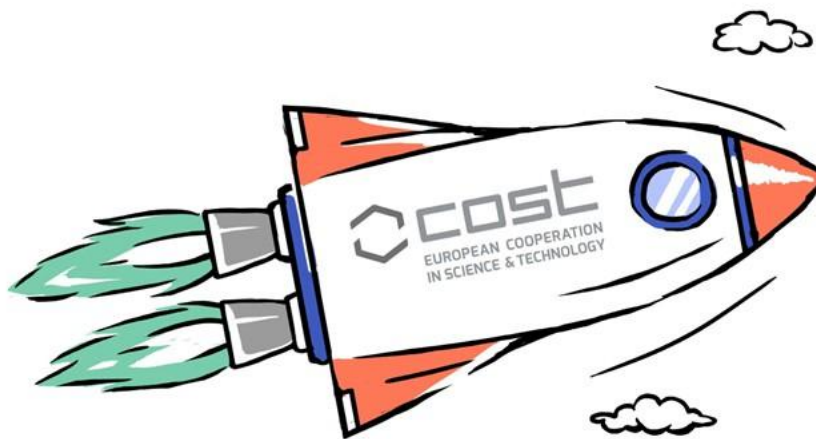
Key Strategic Themes:

1. **Fe-S Biogenesis as a Drug Target:** The mycobacterial SUF system and mitochondrial iron metabolism in parasites represent validated targets for antimicrobial development, with mitoDFO and SUF inhibitors as promising clinical candidates.
2. **Protein Engineering for Therapeutic Enzymes:** The iCARE platform demonstrates that deep mechanistic understanding of Fe-S enzyme electron transfer can be leveraged to create autonomous therapeutic enzymes with minimized off-target effects.
3. **Nanocage-Based Delivery Systems:** The PINC engineering approach provides a versatile platform for drug delivery and vaccine development, with applications in oncology and infectious diseases.
4. **Broad-Spectrum Antiviral Strategies:** The GC-376 PROTAC platform demonstrates that targeting conserved viral protease structures enables development of antivirals with pan-viral efficacy.
5. **Metalloprotein-Based Therapeutics:** The BV-Cu complex represents a novel class of metallotetrapyrrole therapeutics with distinct mechanisms of action.
6. **Biocompatible Immobilization Platforms:** Strep-MIP technology enables practical application of metalloenzymes in biotechnology, bridging the gap between fundamental research and industrial application.

Proposed Next Steps for WP5:

1. **SUF Inhibitor Development:** WP5 will prioritize high-throughput screening and structure-based drug design targeting the SufS-SufU interface in *M. tuberculosis*, building on the mechanistic insights from WP3.
2. **mitoDFO Preclinical Development:** We will advance mitoDFO through preclinical testing, including pharmacokinetic studies, toxicity assessment, and efficacy evaluation in animal models of leishmaniasis and fungal infections.
3. **iCARE Therapeutic Translation:** Building on the successful proof-of-concept, WP5 will initiate studies to evaluate iCARE delivery strategies (e.g., mRNA-based, viral vector) for in vivo antiviral applications.
4. **PROTAC Optimization:** We will optimize the GC-376 PROTAC for improved potency, selectivity, and pharmacokinetic properties, with the goal of developing a pan-viral therapeutic candidate.
5. **PINC Platform Expansion:** WP5 will explore the application of PINCs for targeted drug delivery to specific cell types via surface receptor ligands and for development of combination therapies.
6. **BV-Cu Mechanism Elucidation:** We will pursue detailed mechanistic studies to identify the molecular targets of BV-Cu and evaluate its efficacy in animal models of cancer.
7. **Cross-WP Collaboration:** WP5 will collaborate with WG4 to apply insights from Fe-S cluster gas sensing (mitoNEET) toward developing novel therapeutics for inflammatory and age-related diseases.

Disclaimer: *This report is a synthesis document prepared for COST Action Working Group 5 based on the provided abstracts. Specific grant numbers and author lists are omitted for brevity but are available in the original publications.*



Deliverables 20-21

Publications

1. Constructing safe and highly potent antiviral neutralising nanodevices using a ferritin-based plug-and-play molecular assembly platform, ChemRxiv, DOI: <https://doi.org/10.26434/chemrxiv.15008962/v1>
2. Iron–sulfur proteins in mycobacteria: master regulators of physiology and pathogenesis, Trends in Microbiology (2026) Volume 34, Issue 1p62-75. DOI: [10.1016/j.tim.2025.06.014](https://doi.org/10.1016/j.tim.2025.06.014)
3. Interaction studies of biliverdin-Cu complex formation using XANES, XRF, and DFT analyses and its effect on cytotoxicity in glioblastoma cell line (U251), Archives of Biochemistry and Biophysics (2025) 770, 110466. DOI: [10.1016/j.abb.2025.110466](https://doi.org/10.1016/j.abb.2025.110466)
4. A Strep-Tag Imprinted Polymer Platform for Heterogenous Bio(electro)catalysis, Angewandte Chemie (2024) Volume63, Issue47, e202408979. DOI: <https://doi.org/10.1002/anie.202408979>
5. LIGANDS BINDING [Fe₂-S₂] CLUSTER PROTEINS: DFT APPROACH FOR DRUG-LIKENESS ASSESSMENT, U.P.B. Sci. Bull., (2025) Series B, Vol. 87, Iss. 2.
6. 2 Metal Ion Catalysis in the, Book Chapter, DOI: 10.1201/9781003459910-2
7. A Versatile Virus-Mimetic Engineering Approach for Concurrent Protein Nanocage Surface-Functionalization and Cargo Encapsulation, Small (2024) 20, 2310913. DOI: <https://doi.org/10.1002/sml.202310913>
8. A Structural Investigation of the Interaction between a GC-376-Based Peptidomimetic PROTAC and Its Precursor with the Viral Main Protease of Coxsackievirus B3, Biomolecules 2024, 14(10), 1260; <https://doi.org/10.3390/biom14101260>
9. Chelation of Mitochondrial Iron as an Antiparasitic Strategy, ACS Infect. Dis. (2024) 10 (2): 676–687. DOI: <https://doi.org/10.1021/acsinfecdis.3c00529>
10. Multi-structural molecular docking (MOD) combined with molecular dynamics reveal the structural requirements of designing broad-spectrum inhibitors of SARS-CoV-2 entry to host cells, Scientific Reports (2023) volume 13, Article number: 16387. DOI: <https://doi.org/10.1038/s41598-023-42015-2>
11. VITAS, a sensitive in vivo selection assay to discover enzymes producing antiviral natural products, Chem. Commun. (2023) 59 (36): 5419–5422. DOI: <https://doi.org/10.1039/d3cc00638g>
12. New Apoptosis Inducers Containing Anti-inflammatory Drugs and Pnictogen Derivatives: A New Strategy in the Development of Mitochondrial Targeting Chemotherapeutics, J. Med. Chem. (2023) 66 (6): 4131–4149. DOI: <https://doi.org/10.1021/acs.jmedchem.2c02126>

