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In Silico Evidence for Enterobactin-Facilitated Ferric Iron Import into Mitochondria via ATP Synthase Alpha Subunit Binding

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Abstract

Research on mitochondrial iron metabolism has largely focused on Fe(II), as it is relatively soluble and readily available under physiological conditions. By contrast, the role of Fe(III) remains less well understood. Its poor solubility and tendency to form potentially harmful ferric hydroxides mean that it must remain bound to suitable ligands or chaperones during transport. Canonically, iron is delivered to cells via transferrin (Tf), which binds the transferrin receptor (TfR1) for receptor-mediated endocytosis. Acidification of endosomes releases Fe(III), which is then reduced to Fe(II) by STEAP3 and transported into the cytosol by DMT1, forming a labile iron pool for mitochondrial use. However, the direct trafficking of Fe(III) into mitochondria has remained unexplored. Here, we propose a potential model for Fe(III) uptake into mitochondria through enterobactin, a bacterial siderophore known to influence host iron metabolism. Using in silico docking with AutoDock Vina and AlphaFold2-derived mitochondrial F-type ATP synthase structures, we observe that Fe(III)-enterobactin complexes yield docking scores comparable to Fe(II)-enterobactin complexes at the ATP synthase alpha subunit. In addition, enterobactin itself adopts compatible binding poses at the protein surface. These observations are consistent with a possible interaction framework but do not establish a dissociative mechanism for iron delivery within the mitochondrial matrix. These docking analyses support the structural plausibility of an enterobactin-associated ferric iron interaction with the mitochondrial ATP synthase alpha subunit. Although these findings do not establish a specific transport mechanism, they raise the possibility of an alternative route for mitochondrial iron

trafficking. Further experimental work is needed to determine whether this pathway operates in cells and how it contributes to iron homeostasis. More broadly, the proposed model suggests a possible connection between microbial siderophores and mitochondrial iron handling, with potential physiological and pathological relevance.

Keywords: Mitochondrial iron metabolism; Enterobactin; Chelation; Adenosine triphosphate; Fe(III) trafficking; Molecular docking

1. Introduction

Mitochondria are best known for producing cellular energy, but they also play a central role in iron metabolism. Iron can readily switch between oxidation states, making it well suited to the redox reactions that support energy production through haem- and iron–sulfur cluster-dependent electron transport. Mitochondria are the sole location for haem biosynthesis and a principal source of ISCs, both of which are crucial for a wide range of metabolic and regulatory functions (1). Perturbations in mitochondrial iron homeostasis can trigger cell death. An example in this regard is ironomycin, a small molecule that depletes mitochondrial iron, (2). Frataxin (FXN), a small acidic protein with intrinsically disordered regions is central to mitochondrial iron regulation. FXN binds both of the Fe(II) and Fe(III) states of iron, regulates ISC biogenesis, and is involved in ferroptosis, a form of iron-dependent cell death implicated in multiple diseases (3–5).

The essential role of iron in biological systems is balanced by its potential toxicity. Iron in its ferrous form [Fe(II)] is soluble and bioavailable, but it can be easily oxidised in physiological oxygen to ferric iron [Fe(III)]. Ferric iron hydrolyses into insoluble ferric hydroxides. To mitigate this, organisms have evolved highly regulated systems for iron acquisition, transport, and storage in nontoxic forms (6).

Transferrin (Tf) moves iron in the blood under normal physiological conditions. It does this by delivering Fe(III) to cells through receptor-mediated endocytosis via transferrin receptor 1 (TfR1). Proton pumps make endosomes more acidic, which lowers Tf's ability to bind iron. Acidification of the endosome releases Fe(III) from transferrin, after which STEAP-family ferrireductases reduce it to Fe(II). Divalent metal transporter 1 (DMT1) then transports Fe(II) across the endosomal membrane into the cytosol, where it contributes to the labile iron pool available for mitochondrial uptake. Iron enters mitochondria primarily as Fe(II), with mitoferrins among the best-characterised transporters of the inner mitochondrial membrane, although several aspects of this process remain unresolved. Once inside the mitochondrial matrix, iron is used for haem synthesis and iron–sulfur cluster assembly or

stored in mitochondrial ferritin. Because mitochondria are also a major source of reactive oxygen species, mitochondrial iron must be tightly controlled to limit iron-driven oxidative damage (6,7).

Nevertheless, a model based solely on the uptake of free Fe(II) may not capture the full complexity of mitochondrial iron trafficking. Under aerobic physiological conditions, a substantial proportion of iron exists as Fe(III), depending on the local redox and chemical environment (6). Fe(III) is poorly soluble and readily forms insoluble hydroxides, so it generally remains bioavailable only when coordinated by suitable ligands. Siderophores provide an especially effective means of achieving this. Enterobactin, for example, binds Fe(III) with extremely high affinity, raising the possibility that some ferric iron could be trafficked in a ligand-bound form rather than first being released and reduced to free Fe(II). Such a route could be particularly relevant at host–microbe interfaces, where microbial siderophores are present (6,8).

Evidence from Qi and Han (2018) lends support to this possibility. Their study showed that enterobactin, a bacterial siderophore produced by members of the gut microbiota, promotes mitochondrial iron uptake in *C. elegans* and mammalian cells. Remarkably, this mechanism appears to occur inside mitochondria and independently of ATP synthase (F-type) catalytic activity. Enterobactin binds to the alpha subunit of the mitochondrial ATPase, promoting the labile iron pool and organismal growth. Originally considered detrimental to host iron availability, these findings instead highlight a novel commensal-host interaction and suggest that enterobactin may act as an iron delivery vector for mitochondria (9,10).

In light of these insights, we propose and explore an alternative model for mitochondrial iron uptake that includes direct trafficking of Fe(III) into mitochondria in the form of enterobactin-bound complexes. Using a structure-based in silico docking approach, we examine whether Fe(III)- and Fe(II)-enterobactin species can adopt compatible interaction poses with the ATP synthase alpha subunit, with the aim of generating a testable structural hypothesis for a potential noncanonical route of mitochondrial iron handling.

2. Materials and Methods

All schematic representations are conceptual and not intended to reflect structural scale or precise molecular architecture.

All molecular docking simulations were conducted using AutoDock Vina (11,12). The input structures were obtained from curated public databases: iron species [Fe(II) and Fe(III)] and enterobactin were downloaded from PubChem in SDF format, while protein structures were retrieved as PDB files from two sources: (1) the AlphaFold2-predicted human mitochondrial F₁ ATPase (F-type ATP synthase) alpha subunit (P25705-ATPA_HUMAN) (13,14), which was selected as the primary human receptor because this study specifically investigated the human ATP synthase alpha subunit implicated in enterobactin interaction by Qi and Han (9). The AlphaFold2 model provided a complete structure of the isolated human alpha subunit suitable for whole-surface molecular docking analyses, whereas available experimental human ATP synthase structures predominantly represent multimeric complexes rather than isolated alpha subunits. Protein structures were also obtained from the RCSB Protein Data Bank for bovine (PDB ID: 1BMF) and yeast (PDB ID: 2HLD) mitochondrial F₁ ATPases. To assess structural consistency across different species, these experimentally resolved ATP synthase structures were included alongside the AlphaFold2-predicted human model. This allowed assessing whether the predicted enterobactin interactions were qualitatively conserved across species and did not depend on a single structural model.

For the AlphaFold2-predicted regions, confidence metrics such as pLDDT scores were not analysed in this study, and therefore the docking interpretation does not account for local structural uncertainty. The work focuses on ligand–protein docking using established AutoDock Vina workflows.

File format conversions were carried out using Open Babel GUI (15), which allowed transformation of SDF and PDB files into the PDBQT format required by AutoDock Vina. During pre-processing, all water molecules were removed from protein structures, polar hydrogen atoms were added, missing atoms were repaired and Kollman charges were assigned to receptor proteins. The larger molecular structure (typically the protein) was designated as the receptor, while the smaller molecular entity (metal ion, enterobactin, or complex) was treated as the ligand.

Grid box dimensions were configured to cover the full surface of the receptor for each docking experiment, which enabled the unbiased sampling of potential binding sites. Visualization and analysis of the docking output files, containing binding poses and predicted binding affinities, was done using PyMOL (16) for initial inspection and UCSF ChimeraX (version 1.12) (17) for subsequent structural

visualization and preparation of publication-quality figures. LigPlot+ v2.2 (20), licensed for academic use by EMBL-EBI, was used to generate 2D interaction diagrams highlighting hydrogen bonds and hydrophobic contacts, in order to examine specific ligand-receptor interactions.

This *in silico* approach was applied consistently across all studied systems, including Fe(II)/Fe(III) interactions with enterobactin and their subsequent binding to the mitochondrial ATPase alpha subunit in human, bovine, and yeast models.

3. AutoDock Vina does not explicitly parameterize transition metal coordination chemistry, particularly for Fe(III), which exhibits specific coordination geometries and ligand field effects. In this study, metal–ligand interactions were treated within the limitations of the docking framework, and therefore the results do not capture the true energetics of Fe–enterobactin chelation. Accordingly, docking outputs are interpreted in terms of protein–ligand surface compatibility rather than intrinsic metal–ligand stability. To assess the Fe(III)–enterobactin coordination geometry represented by the docking configurations, the six shortest distances between Fe(III) and enterobactin oxygen atoms were compared with experimentally determined ferric-enterobactin structures. The single-crystal X-ray structure of ferric enterobactin reported by Johnstone and Nolan (18) was used as a direct chemical reference for Fe(III) coordination geometry. In addition, the protein-bound ferric-enterobactin structures deposited as PDB entries 6I2J and 6R1F (19) were examined as structural references. This comparison was used to assess whether the Vina-generated configurations reproduced the experimentally observed Fe(III) coordination environment and was not used to derive metal-binding energies or rescore the docking results (Table S1).

4. Results

3.1 Initial docking of iron-enterobactin complexes to the ATPase alpha subunit

To explore the binding preferences of Fe(II) and Fe(III) with the mitochondrial ATPase alpha subunit, four docking strategies were employed using AutoDock Vina. The AlphaFold2-predicted structure of the human ATP synthase alpha subunit (P25705-ATPA_HUMAN) was used as the receptor. The approaches involved: (1) docking Fe(II) into an enterobactin-alpha complex, (2) forming an Fe(II)-enterobactin complex followed by docking to alpha, and (3–4) analogous methods for Fe(III). Additionally, in approaches 2 and 4, two Fe–enterobactin configurations generated from the initial metal–enterobactin docking were subsequently evaluated in docking against the ATP synthase alpha subunit..

Table 1 summarizes the docking workflows, while Table 2 presents the corresponding binding affinities. Among the results, Fe(II)-enterobactin docked to the alpha subunit (Approach 2a) and

Fe(III) docked into the enterobactin-alpha complex (Approach 3) both yielded the most negative binding affinities, at -9.4 kcal/mol. These comparable values indicate that both oxidation states of iron are compatible with similar docking poses involving enterobactin and the alpha subunit. Notably, these results point to structural compatibility rather than confirming a stable ternary complex *in vivo*. Docking algorithms cannot explicitly account for the aqueous and alkaline environment of the mitochondrial matrix, which is a limitation detailed in Figure S1. All calculated binding affinities are captured in Figure S2; noticeably, Approach 2b yielded the least favourable docking scores among the tested approaches.

Figures S3–S6 break down the representative docking poses and interaction diagrams. Looking specifically at Figure S3C, enterobactin establishes clear hydrogen bonds with three key residues of the ATPase alpha subunit: Ser184, Asp390, and Arg416. To provide structural support for these outputs, LigPlot+ diagrams map the non-ionic contacts (indicated by red arcs).

The analyses suggest that both free enterobactin and its iron-bound forms can physically fit the ATP synthase alpha subunit. There is a crucial caveat, however. Docking scores for isolated metal–ligand complexes do not capture intrinsic chelation stability. Hence, we avoided using these scores to infer true metal-binding energetics in this study.

The Fe(III) coordination geometry represented by the docking workflow was additionally compared with experimental structural data. The single-crystal structure of ferric enterobactin reported by Johnstone and Nolan establishes a hexadentate Fe(III) coordination environment formed by the three bidentate catecholate groups, with Fe–O bond lengths of approximately 1.965 – 2.041 Å and a distorted coordination geometry intermediate between ideal octahedral and trigonal-prismatic geometries. Consistent with a compact six-coordinate environment, the six nearest enterobactin oxygen atoms in the protein-bound structures 6I2J and 6R1F were located 2.129 – 2.270 Å and 1.925 – 2.175 Å from Fe, respectively. In comparison, the six nearest Fe–O distances in the two Vina-generated configurations were 2.438 , 2.605 , 2.708 , 3.111 , 3.603 and 4.572 Å, and 2.391 , 2.398 , 3.364 , 3.816 , 4.577 and 5.157 Å, respectively (Table S1). Thus, the Vina-generated configurations did not reproduce the compact six-coordinate Fe(III) environment observed experimentally.

4.2 Cross-species docking with bovine and yeast mitochondrial ATPases

To examine whether similar docking patterns were observed across additional structural models, Fe(III)-enterobactin complex was docked into the mitochondrial F1 ATPases of cattle (PDB ID: 1bmf) and yeast (PDB ID: 2HLD). The most favorable docking scores for each species are summarized in

Table 3.

The bovine and yeast models yielded slightly more negative scores compared to the human model; however, these differences fall within the uncertainty of docking scoring functions and should not be interpreted as quantitative differences in binding strength.

In bovine ATPase, the Fe(III)-enterobactin complex bound to residues from both the alpha (C chain) and beta (D chain) subunits. In yeast, the complex interacted with the L, P, and M chains (alpha and beta subunits). Figures S7 and S8 show the docking conformations and corresponding interaction networks derived from LigPlot+.

For example, in bovine ATPase, the strongest interactions included hydrogen bonds with Tyr381 and Arg408 of the D chain (Figure S7). In yeast ATPase, Fe(III)-enterobactin interacted with Gln332, Asp335, and Arg293 of the L chain, Leu3 and Asn265 of the P chain and Asp319 of the M chain. (Figure S8).

These results are consistent with the possibility that enterobactin-bound iron can adopt compatible interaction poses with mitochondrial ATPases across species, binding both alpha and beta subunits in different configurations, without implying functional or catalytic relevance of these interactions.

3.3 Hypothetical outcomes following Fe(III)–enterobactin association with ATP synthase

In certain docking conformations, particularly those observed in Approach 3, the Fe(III) ion adopts positions in closer spatial proximity to both enterobactin and key interacting residues compared to other docking outputs. While these observations arise from static docking poses and do not reflect thermodynamic stability, they identify alternative spatial arrangements of Fe(III), enterobactin, and nearby protein residues. The functional significance of these geometries, including any relationship to iron reduction or ligand dissociation, cannot be determined from the present docking analysis..

The docking results are consistent with the structural possibility of Fe(III)–enterobactin association with the ATP synthase alpha subunit. Whether such an association occurs in mitochondria or is followed by iron reduction, dissociation, or transport cannot be determined from the present analysis.. However, these observations are based on static docking poses and do not provide direct evidence for thermodynamic favorability or mechanistic coupling to ATP hydrolysis. The involvement of ATP-dependent processes therefore remains speculative and is presented here as a working model rather than a demonstrated mechanism. Importantly, these docking results do not account for mitochondrial pH, redox conditions, or solvent effects, all of which are expected to influence iron coordination chemistry and protein–ligand interactions in vivo.

For hypothesis generation, three possible outcomes following the predicted protein–ligand association are considered (Figure 1)::

1. Fe(III) is reduced to Fe(II) while still bound to the enterobactin-alpha complex.
2. Fe(III)-enterobactin dissociates from the protein, then undergoes reduction.
3. Fe(III) dissociates from both enterobactin and the protein before reduction (considered least likely due to Fe(III)'s insolubility and toxicity).

. The current docking analysis does not provide evidence for energetic coupling or mechanistic involvement of ATP hydrolysis. These observations are based on static docking poses and do not account for protein dynamics or solvent effects and therefore should be interpreted as indicative of structural compatibility rather than evidence of stable complex formation.

4. Discussion

Docking provides static structural snapshots and does not capture conformational flexibility or time-dependent stability of the complexes. Hence, the values discussed in this manuscript should be interpreted qualitatively, as docking scores do not represent true thermodynamic parameters. The structural comparison with experimentally determined ferric-enterobactin provides a direct illustration of this limitation. Experimental structures show Fe(III) coordinated by six enterobactin oxygen donors in a compact coordination environment, whereas the Fe(III) positions generated by the Vina workflow did not reproduce this geometry. The docking-generated Fe(III)–enterobactin configurations therefore should not be interpreted as chemically validated models of ferric-enterobactin chelation. In particular, the associated docking scores cannot establish Fe(III) coordination stability or chelation energetics. The protein-docking results are consequently interpreted only as exploratory assessments of spatial compatibility for the configurations sampled.

Our docking analyses indicate that enterobactin, both in its free and iron-bound forms, can adopt structurally compatible poses at the mitochondrial ATP synthase alpha subunit. While these findings suggest the plausibility of an interaction, they do not establish stable binding or a functional transport mechanism. Instead, the results provide a structural basis for a working hypothesis in which enterobactin-associated ferric iron could interact with mitochondrial ATP synthase, without establishing the stability, specificity, or functional consequences of this predicted association.. We note that the present study does not include systematic specificity controls (e.g., alternative proteins or ligands), and therefore the observed interactions should not be interpreted as uniquely selective for

ATP synthase. Taken together, the docking analyses identify structurally compatible predicted poses at the ATP synthase alpha subunit, but do not independently establish stable or selective binding..

4.1 Potential candidates for mitochondrial Fe(III) reduction

If enterobactin-associated Fe(III) reaches the mitochondrial matrix, subsequent reduction to Fe(II) could permit its incorporation into established mitochondrial iron-utilisation pathways. The present study does not establish such mitochondrial entry or identify the reducing system that would act on enterobactin-associated iron. Several mitochondrial redox-active proteins can nevertheless be considered as hypothetical candidates for this unresolved reduction step, as illustrated in Figure 2..

Frataxin (FXN) is known to bind to both Fe(III) and Fe(II), in addition to acting as a storage protein or chaperone for ISC biosynthesis (21). The ability of FXN to sequester Fe(III) in less reactive forms and deliver Fe(II) to partner enzymes places it as a potential candidate for driving intramitochondrial Fe(III) reduction. Cytochrome b reductase 1 (CYBRD1), traditionally known to be involved in duodenal iron absorption, has components that are homologous within the mitochondrial complex III, thereby raising the possibility of a cytochrome-based mechanism that contributes to matrix-localized Fe(III) reduction (22).

Additionally, the electron-donating capacity of ferredoxins, such as mammalian FDX1 and FDX2, could potentially support Fe(III) reduction as part of the proposed model. Ferredoxins participate in electron transfer for ISC assembly and are implicated in redox signalling within the mitochondrial matrix (23-25).

A known mitochondrial ferric chelate reductase, plant FRO3, has structural homologues in humans with their precise subcellular localisation being unclear. The possible role of this reductase in the mammalian mitochondrial redox network necessitates further investigation (26). Collectively, these proteins illustrate potential sources of mitochondrial reducing activity; however, their involvement in the reduction of enterobactin-associated Fe(III) has not been established.(27).

The authors acknowledge that this study has limitations inherent to molecular docking. The explicit modelling of transition metal coordination chemistry cannot be done by AutoDock Vina, hence not accurately capturing the thermodynamics of Fe(III)-enterobactin chelation. In addition, docking does not account for protein dynamics, the mitochondrial environmental or solvent effects, providing only static snapshots. Hence, the interactions predicted should be interpreted as plausible from a structural perspective rather than direct evidence of stable binding or an explicit transport mechanism that has been validated (28-31,33,34). To establish the physiological relevance of the proposed model, further experimental validation would be required.

A major unresolved question is whether and in what chemical form enterobactin-associated iron can access mitochondria. Enterobactin is a bacterial siderophore, and its intracellular trafficking in mammalian systems remains incompletely understood. Potential barriers include transport across cellular and mitochondrial membranes, as well as the stability and concentration of siderophore-bound iron within the host environment.

The mechanisms that govern the localisation and transport of enterobactin remain to be fully elucidated despite prior studies suggesting a potential role for enterobactin in mitochondrial iron uptake (9,32).

The model proposed in this manuscript should be interpreted within the broader context of biological and computational uncertainty. Molecular dynamics simulations could in principle provide information on the persistence and conformational behaviour of protein–ligand configurations; however, rigorous simulation of Fe(III)–enterobactin requires an appropriately parameterized treatment of the Fe(III) coordination environment. Conventional biomolecular force fields do not intrinsically provide a validated description of transition-metal coordination, and simulations of metal–chelator systems can require metal-specific bonded or non-bonded models, appropriate charge derivation, and validation against experimental or quantum-chemical structural data (35,36). This consideration is particularly important here because the structural comparison performed in the present revision showed that the Vina-generated Fe(III)–enterobactin configurations do not reproduce the experimentally observed compact six-coordinate Fe(III) environment (Table S1). Performing conventional MD directly from these docking-derived configurations would therefore not provide a rigorous validation of Fe(III)–enterobactin stability without first establishing and validating an appropriate metal-coordination model. Such parameter development and validation are beyond the scope of the present hypothesis-generating docking study. Instead, we have provided direct structural benchmarking against experimentally determined ferric-enterobactin structures and explicitly restricted interpretation of the docking results to exploratory spatial compatibility. Further studies using validated Fe(III)–catecholate parameters or appropriate QM/MM approaches will be required to examine the dynamics and stability of these systems.

5. Conclusion

Our docking analyses identify structurally compatible predicted poses of enterobactin-associated ferric iron at mitochondrial ATP synthase. These calculations do not establish stable or selective binding, mitochondrial transport, or a mechanism of iron release or reduction. Rather, they provide a structural hypothesis that can be tested experimentally and through further appropriately parameterized computational studies. Together with previous evidence linking enterobactin to mitochondrial iron uptake, the present findings motivate investigation of whether enterobactin-associated Fe(III) interacts with ATP synthase under physiological conditions. Biochemical binding assays, mitochondrial import experiments, and genetic studies will be required to establish the existence and functional significance of such an interaction.

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AI Declaration

The authors acknowledge the use of ChatGPT (OpenAI) as a large language model for language editing, improving readability, and refining the clarity of expression. All AI-assisted suggestions were reviewed, verified, and revised by the authors. The scientific content, interpretation, and conclusions were developed independently by the authors.

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Conflicts of Interest

The authors declare no conflicts of interests whatsoever.

List of Abbreviations

- **ATP:** Adenosine triphosphate
- **CYBRD1:** Cytochrome b Reductase 1
- **DMT1:** Divalent metal transporter 1
- **FDX:** Ferredoxin
- **FDXR–FDX:** Ferredoxin Reductase–Ferredoxin
- **FXN:** Frataxin
- **ISC:** Iron–sulfur cluster
- **pLDDT:** predicted Local Distance Difference Test
- **PDB:** Protein Data Bank
- **ROS:** Reactive oxygen species
- **SDF:** Structure Data File
- **STEAP3:** Six-Transmembrane Epithelial Antigen of the Prostate 3
- **Tf:** Transferrin
- **TfR1:** Transferrin receptor 1

Author Contributions

Conceptualization: G.N.P., A.P.; Methodology: A.D.; Software: A.D.; Validation: A.D.; Formal analysis: A.D.; Investigation: A.D.; Resources: A.D.; Data curation: A.D.; Visualization: A.D.; Writing – original draft: A.D.; Writing – review and editing: A.D., A.P., G.N.P.; Supervision: G.N.P., A.P.; Funding acquisition: A.P., G.N.P. All authors reviewed and approved the final manuscript.

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Table 1: The various docking approaches used in this study.

Approach	First docking	Second docking
1	Ent-alpha	Fe2+-(Ent-alpha)
2a	Fe2+-Ent	Alpha-(Fe2+-Ent), most favourable docking pose from first docking
2b	Fe2+-Ent	Alpha-(Fe2+-Ent), second-ranked docking pose from first docking
3	Ent-alpha	Fe3+-(Ent-alpha)
4a	Fe3+-Ent	Alpha-(Fe3+-Ent), most favourable docking pose from first docking
4b	Fe3+-Ent	Alpha-(Fe3+-Ent), second-ranked docking pose from first docking

Table 2 : The values of binding affinities (in kcal/mol) obtained from Autodock Vina for the approaches outlines above in Table 1. The primary focus is on the most negative values.

S. No	Approach 1	Approach 2a	Approach 2b	Approach 3	Approach 4a	Approach 4b
1	-8.9	-9.4	-8.7	-9.4	-8.6	-8.4
2	-8.8	-8.9	-8.3	-8.9	-8.5	-8.4
3	-8.6	-8.7	-8.2	-8.3	-8.2	-8.4
4	-8.4	-8.5	-7.8	-8.2	-8.1	-8.3
5	-8.3	-8.4	-7.7	-8.2	-7.8	-8.2
6	-8.2	-8.2	-7.6	-8.2	-7.7	-8.1
7	-8.2	-8.1	-7.4	-8.1	-7.7	-8.1
8	-8.1	-8.1	-7.3	-8.0	-7.6	-8.0
9	-7.9	-8.1	-7.2	-8.0	-7.6	-8.0

Table 3 : Comparative binding affinity values in kcal/mol for the most favourable docking poses for binding with Fe(III)-enterobactin among different organisms.

ATPase	Most favourable docking pose (Fe³⁺-Ent)
Human alpha subunit from AlphaFold2 (P25705-ATPA_HUMAN)	-8.6
Bovine whole mitochondrial F1 ATPase (PDB ID 1bmf)	-9.7
Yeast mitochondrial ATPase (PDB ID 2HLD)	-10.2

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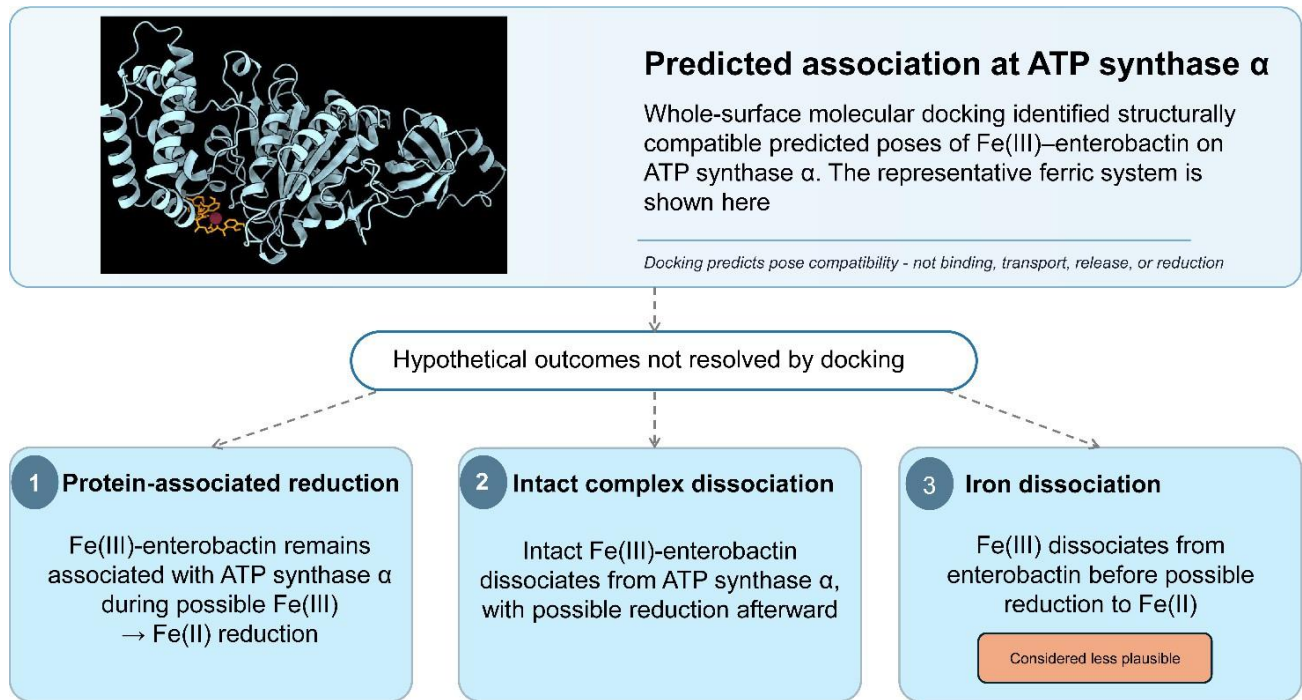


Figure 1

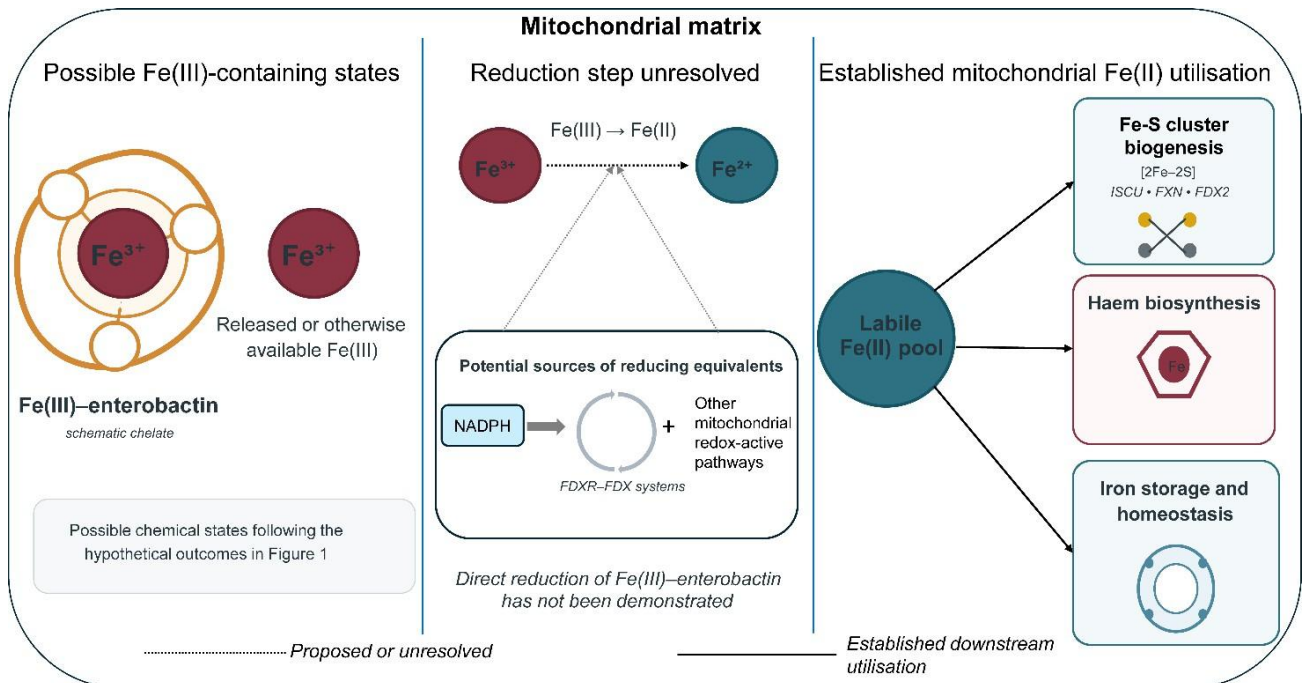


Figure 2

Figure 1. Hypothetical outcomes following the predicted association of Fe(III)–enterobactin with mitochondrial ATP synthase α .

Whole-surface molecular docking identified structurally compatible predicted poses of Fe(III)–enterobactin on ATP synthase α . Three hypothetical outcomes following this predicted association are considered: (1) reduction of Fe(III) to Fe(II) while enterobactin remains associated with ATP synthase α ; (2) dissociation of intact Fe(III)–enterobactin from ATP synthase α , followed by possible reduction of Fe(III) to Fe(II); and (3) release of Fe(III) from enterobactin before its possible reduction to Fe(II). Outcome 3 is considered less plausible because of the high affinity of enterobactin for Fe(III). The pale-blue panel represents the structural inference derived from docking, whereas grey dashed arrows indicate hypothetical outcomes that were neither examined nor resolved by docking. Although Fe(II)-containing systems were also evaluated computationally, this schematic focuses on Fe(III)–enterobactin because the proposed downstream model concerns the possible release and reduction of ferric iron. The schematic is conceptual, is not drawn to scale, and does not represent a demonstrated transport, dissociation, iron-release, or reduction mechanism.

Figure 2. Proposed relationship between unresolved mitochondrial Fe(III) reduction and downstream iron utilisation.

Following the hypothetical outcomes presented in Figure 1, mitochondrial Fe(III) may remain associated with enterobactin or become available in another chemical form. Its reduction to Fe(II) is depicted as unresolved because neither the relevant iron-containing substrate nor the responsible reducing system was established in this study. Mitochondrial FDXR–FDX systems and other redox-active pathways may provide reducing equivalents, but their involvement in enterobactin-associated iron reduction remains hypothetical. Bioavailable Fe(II) may subsequently enter established mitochondrial pathways supporting iron–sulfur cluster biogenesis, haem biosynthesis, and iron storage or homeostasis. Dotted connectors represent proposed or unresolved relationships, whereas solid arrows represent established downstream utilisation of mitochondrial iron. The schematic is conceptual and is not drawn to scale.