

# Chemistry and Biology of Ferritin

Marina Plays<sup>1,2,3,4</sup>, Sebastian Müller<sup>1,2,3,4\*</sup>, Raphaël Rodriguez<sup>1,2,3,4\*</sup>

<sup>1</sup> Chemical Biology of Cancer Laboratory, Institut Curie, 26 rue d'Ulm, 75005 Paris, France

<sup>2</sup> CNRS UMR 3666

<sup>3</sup> INSERM U1143

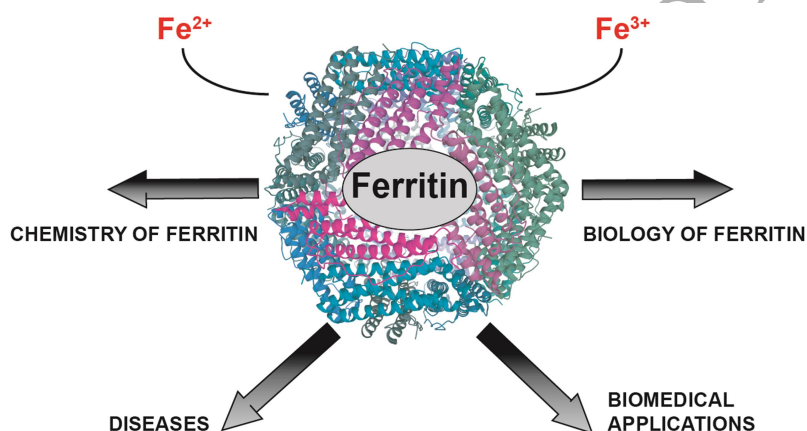
<sup>4</sup> PSL Université Paris, France

\*correspondance to: raphael.rodriguez@curie.fr, sebastian.muller@curie.fr

## Abstract

Iron is an essential element required by cells and has been described as a key player in ferroptosis. Ferritin operates as a fundamental iron storage protein in cells forming multimeric assemblies with crystalline iron cores. We discuss the latest findings on ferritin structure and activity and its link to cell metabolism and ferroptosis. The chemistry of iron, including its oxidations states, is important for its biological functions, its reactivity and the biology of ferritin. Ferritin can be localized in different cellular compartments and secreted by cells with a variety of functions depending on its spatial context. Here, we discuss how cellular ferritin localization is tightly linked to its function in a tissue-specific manner, and how impairment of iron homeostasis is implicated in diseases including cancer and COVID-19. Ferritin is a potential biomarker and we discuss latest research where it has been employed for imaging purposes and drug delivery.

## Graphical abstract



## Introduction

Iron is an essential element for living systems, where it is crucial for oxygen transport, cellular energy production and importantly can either serve as a cofactor, or catalyst involving enzymatic reactions [1]. In humans, 70% of iron is incorporated into hemoglobin to carry oxygen in red blood cells. Because of its specific electron configuration, iron can act as a redox catalyst, for instance in iron-dependent histone demethylases [2]. Moreover, it can be found in iron-sulfur (Fe-S) clusters and heme groups. Although iron is essential for life,

excess cellular iron is toxic as labile iron can lead to oxidative stress, so the cell has evolved an intricate system to balance cellular iron import, export and storage in order to regulate iron homeostasis. Ferritin is a multimeric protein forming a nanocage structure, which can contain up to around 4500-5000 iron atoms [3, 4]. It is ubiquitous in all forms of cellular life, with the exception of yeast [5], and has wide-ranging functions besides iron storage, including maintenance of cellular iron concentrations, iron sequestration from invading pathogens, and oxidative stress protection [6]. Ferritin has been characterized as a cytosolic protein, but it is also present in other cell compartments, such as the nucleus, mitochondria and lysosomes, and a small part of ferritin is located in the serum. This review reports the most recent findings on ferritin structure and functions, and highlights new knowledge on the localization and function of ferritin in different cell compartments, linking its functions to iron homeostasis with spatial resolution. We also discuss the role of ferritin in a form of programmed cell death termed ferroptosis [7], an iron-dependent programmed cell death [8], which has important implications in diseases ranging from cancer [9] to Alzheimer's disease. Furthermore, we illustrate the implication of ferritin in various forms of diseases such as cancer, coronavirus disease 2019 (COVID-19), hyperferritinemia, kidney disease as well as its use as a diagnostic and prognostic marker. Finally, we describe ferritin-based bio-nanotechnologies and their applications.

## **Ferritin structure and activity**

### **Ferritin genes and ferritin supramolecular assembly**

The ferritin protein superfamily can be divided into three major subclasses: the classical ferritins (Ftn), the heme-containing bacterioferritins (Bfr) and the DNA-binding proteins from starved cells (Dps). Ftn and Bfr are made of 24 subunits, whereas Dps are smaller with 12 subunits. Ftn is found in plants, bacteria and animals, while Bfr and Dps are restricted to prokaryotes. A phylogenetic network analysis based on structure and sequence homology concluded that all three subclasses share a common ancestor [10]. Moreover, a new form of ferritin, the encapsulated ferritin, has recently been discovered in bacteria and archaea [11] and its assembly pathway has been characterized by mass spectrometry [12].

Human ferritin is composed of two different subunits, which have a size of 19 kDa and 21 kDa for the light chain (L-ferritin) and heavy chain (H-ferritin), respectively, and whose genes (FTH1 and FTL) are located on chromosomes 11q and 19q. The two subunits share approximately 55% of sequence homology and have a similar 3D structure, consisting in four parallel and anti-parallel helices (A-D) and a fifth shorter E-helix that forms a 60° angle with the other helices (Figure 1A). Human ferritin subunits co-assemble to form an almost spherical nanocage with inner and outer diameters of 8 nm and 12 nm, respectively. The ferritin nanocage comprises six hydrophobic four-fold (C4) channels, each formed by the E-helices of four monomers, and eight narrow hydrophilic three-fold (C3) channels, which represent pores for iron ions [13] (Figure 1B, C). Importantly, the resulting nanocage that separates the iron core from the outside environment, is very stable between pH 3-9.

The mechanism of ferritin self-assembly has been described using a simple model where only tetramers, hexamers and dodecamers were considered as intermediates [14]. Sato *et al.* showed that *E. coli* ferritin A can dissociate to form dimers at acidic pH, which maintain their native-like secondary and tertiary structures, and can reassemble into 24-mer ferritin when the pH increases [14]. Furthermore, the rate of ferritin assembly increases together with the ionic strength, suggesting that local mainly repulsive electrostatic interactions between assembly units play a role in ferritin self-assembly kinetics [15]. Interestingly, exploiting pH and ionic strength provide the means to trap small molecules into ferritin nanocages for

biotechnology applications such as drug delivery. Using Förster Resonance Energy Transfer enabled to shed light on the mechanism and kinetics of ferritin self-assembly. The formation of H/L-heterodimers was kinetically faster and thermodynamically favored over H/H-homopolymers, which is the initial step of ferritin self-assembly [16]. This explains the heteropolymeric nature of structured ferritin that is favored over the homopolymers. This could argue, that most ferritin multimers are heteropolymers consisting of both ferritin subunits, which would hold true even if the concentration of one of the subunits is much smaller. The H/L ratio is further defined by the abundance and bioavailability of each subunit in the respective cellular compartments.

The technique of cryogenic scanning transmission electron microscopy recently permitted to shed light on the crystallization process of ferritin, which could not be predicted by classical or two-step nucleation theories. Instead, the authors found that amorphous precursors undergo desolvation, leading to aggregates with a continuous increase in both order and density from the surface towards the interior [17]. This discovery can be further exploited, as the crystallization process is important for drug formulation or bionanotechnology applications.

### Iron loading into ferritin and ferroxidase activity

Each ferritin complex can contain between a few hundreds to up to 4500-5000 iron atoms [3, 4]. Importantly, the two ferritin subunits have distinct functions. The H-ferritin subunit has a ferroxidase activity via a dinuclear iron-binding site [18] (Figure 1D), while the L-ferritin subunit lacks this site. Thus, the primary function of the L-ferritin subunit is thought to be iron storage and mineralization within the ferritin multimer core. Iron oxidation and loading into ferritin has been documented, which includes characterization of reaction kinetics *in vitro* [19]. Once  $\text{Fe}^{2+}$  is oxidized into  $\text{Fe}^{3+}$  at ferroxidase site, it moves rapidly towards nucleation sites located on the L-ferritin subunit, subsequently letting the next  $\text{Fe}^{2+}$  ion to access the ferroxidase site. In this way, the presence of the L-ferritin subunit in the heteropolymer enhances the ferroxidase activity of the H-ferritin subunit and thus, this synergistic interaction permits to foster iron oxidation and core formation in H/L-heteropolymers [20]. Thus, the H-subunit plays a major role in the rapid oxidation of  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$ , while the L-subunit clears iron from the center to support iron mineralization and storage [21]. Interestingly, Ferritin nanocages composed only of L-subunits can still oxidize  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$ , but at a much lower rate. Once  $\text{Fe}^{2+}$  ions have reached the catalytic site, they are oxidized by molecular oxygen ( $\text{O}_2$ ) or hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) [22] to produce ferritin-oxide minerals, such as ferrihydrite ( $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ ), magnetite ( $\text{Fe}_3\text{O}_4$ ), maghemite ( $\gamma\text{-Fe}_2\text{O}_3$ ) or hematite ( $\alpha\text{-Fe}_2\text{O}_3$ ), depending on the ferritin bio-environment and iron loading [23, 24]. A mechanism for iron oxidation within the core of ferritin has been proposed, in which Fe(III)-O-Fe(III) resides in the ferroxidase center until it is displaced by a  $\text{Fe}^{2+}$  ion [25]. A recent study using differential pulse voltammetry revealed a stepwise resolution of an iron oxidation mechanism within ferritin. Until now, only C3 channels have been proven to participate in iron loading and ferroxidase activity, but this study gave new evidence that both C3 and C4 channels are functional [26]. During iron loading into ferritin, the mineral core evolves to give different patterns; it grows along the ferritin shell and finally gives either a solid or a hollow structure [3]. It will now be important to investigate how these different iron core structures contribute to ferritin function within the cell.

The C3 channels constitute the main entry pathway for ferrous ions into ferritin nanocages (Figure 1C). Molecular dynamics studies could identify the binding sites of  $\text{Fe}^{2+}$  ions in the C3 channels (Figure 1D) [27], where  $\text{Fe}^{2+}$  ions can be bound simultaneously. When a fourth ion comes close to the entrance of the channel, the deepest ion is released from

the inner binding site and moves to the ferroxidase site. Iron loading into ferritin may depend on the labile iron concentration surrounding the ferritin multimer. A higher labile iron pool (LIP) would influence reaction dynamics and lead to a greater storage of iron into ferritin. Two Glu residues constitute additional  $\text{Fe}^{2+}$  binding sites linking ferritin ion channel exits and the ferroxidase catalytic site or nucleation site (Figure 1D, E) [28]. In a study on *R. catesbeiana* H-ferritin, the authors compared the transit of  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  and concluded that E130 residue in the C3 channel may exert selectivity on the entering metal by influencing the diffusion kinetics of the ions. The metal-ion binding carboxylate of D127 residue dictates the ion channel size and electrostatic properties near the internal exit [29]. A single mutation such as D127E in the sequence of ferritin chains can drastically affect how iron transits through the channel, by altering electrostatic properties. This can considerably reduce enzymatic activity of ferritin and iron mineral core formation inside the multimer [30].

Labile iron can access ferritin channels by diffusion. In humans, chaperones named human poly(rC)-binding protein 1 and 2 (PCBP1 and PCBP2) have been reported to facilitate iron delivery to ferritin [31]. PCBP1 and 2 are a DNA and RNA binding proteins, highly conserved among mammals, widely expressed and located in both the cytosol and the nucleus. Since they function as a cytosolic iron chaperones to deliver iron to ferritin, they play a major role in iron flux within cells [32, 33]. Since both PCBP1 and ferritin are found in the nucleus, it is conceivable that PCBP1 could be responsible for the delivery of iron in the nucleus and possibly also other cell compartments. More research is needed to shed light on these mechanisms. Furthermore, PCBP1 also forms an iron chaperone complex with BOLA Family member 2 (BOLA2) and glutathione (GSH) for the assembly of cytosolic [2Fe-2S] clusters on BOLA2-Glrx3 (glutaredoxin 3) [34]. Therefore, PCBP1 is not only involved in iron storage but also in the synthesis of iron-sulfur clusters, required for the activity of hundreds of proteins within cells. To date, PCBP1 is the only known iron chaperone interacting with ferritin but there might be other unidentified chaperones, loading or unloading iron from ferritin, meriting further research in this area.

## **Ferritin localization**

### **Ferritin in different organs and tissues**

H- and L-ferritin subunits co-assemble into a 24-mer with a specific ratio depending on the tissue type. An early study compared the composition of liver and placenta ferritin and found that the composition of the subunits differs greatly between these different organs [35]. Tissues that have LIPs, such as the muscle tissue or many tissues of the brain, tend to have increased metabolism and energy turnover. These LIPs can pose a risk for the formation of reactive oxygen species (ROS) and these tissues tend to express high levels of H-ferritin. This permits mineralization of excess iron. Evidence for this was reported in mice, where brains deficient of H-ferritin showed normal iron levels, but increased oxidative stress and alterations of other iron homeostasis proteins [36]. Heart tissues also contain increased amounts of H-ferritin, which have been shown to be iron-rich [37]. In organs, such as the liver or the spleen, that are involved in iron storage, high levels of L-ferritin have been observed [38]. Ferritin distribution is not homogenous within the liver, but accumulates in hepatocytes and reticulo-endothelia cells as shown in rats [39]. This suggests that certain regions of the liver are involved in iron storage. Specific transcriptional programs are responsible for the control over H-ferritin expression [40]. Interestingly, the expressions of L-ferritin and H-ferritin are regulated on the transcriptional and post-transcriptional levels in liver and heart [41], and possibly in other organs. Results obtained from transgenic mice expressing H-ferritin from a tetracycline-inducible promoter showed that H-ferritin expression changes

upon iron content and can also induce an iron-depleted phenotype, suggesting that it can actively regulate overall tissue iron balance [42]. Given the interaction between NCOA4 and H-ferritin [43] during ferritinophagy, NCOA4 may favor the degradation of H-ferritin-rich ferritin polymers over L-ferritin polymers. Thus, the expression of H-ferritin and NCOA4 may have an impact on the rate of iron release in different tissues and organs.

### **Ferritin localizations and roles within the cell**

Initially, ferritin was considered predominantly a cytosolic protein with a general iron storage function. Ferritin can also be found in other cell compartments such as the nucleus, mitochondria or lysosomes where it may play various different roles depending on the cellular context (Figure 2). Mitochondrial ferritin can protect cells against ROS and thus against ferroptosis [44]. The expression of L-ferritin and H-ferritin can differ between different cellular compartments. In liver and spleen cells, it was shown that H-ferritin is mainly found in the nucleus, whereas L-ferritin is predominantly found in the cytosol [38]. In addition, specific receptors can take up exogenous ferritin via endocytosis [45, 46]. Further studies are required to investigate how exactly this affects iron homeostasis of cells and how this relates to receptor-mediated iron endocytosis of transferrin or hyaluronates or iron uptake through membrane iron channels. In iron-depleted conditions, ferritin can be found in lysosomal compartments, more specifically in autophagosomes and autolysosomes during a process called ferritinophagy, which generates labile iron from the ferritin stock.

Nuclear ferritin can protect DNA by sequestering iron and preventing uncontrolled Fenton chemistry, or by directly binding to DNA [47, 48]. Nuclear ferritin tends to be mainly H-ferritin and not L-ferritin, which may suggest that the role of nuclear ferritin is not merely for iron storage [49]. Moreover, in corneal epithelial (CE) cells, nuclear ferritin down-regulates the c-Jun N-terminal kinase (JNK) signaling pathway, thus preventing apoptosis in response to cell damage, especially UV radiation. This study on CE cells revealed a feedback loop in which JNK signaling increases the production of nuclear ferritin and, in turn, nuclear ferritin decreases the activity of the JNK signaling pathway [50]. Importantly, recent research showed that nuclear ferritin plays a role in cancer cells plasticity [51]. However, how ferritin translocates from the cytosol to the nucleus is currently unclear. In CE cells, a molecule called ferritoid was able to bind to ferritin and favored its translocation into the nucleus [52]. Nevertheless, ferritoid is specific for CE cells and other molecules may be involved in ferritin nuclear translocation. In addition, this mechanism has not been validated in other cell types to date. Moreover, whether ferritin is transported in a monomeric or multimeric form and where the self-assembling process occurs remain to be defined.

The Ftn family also comprises mitochondrial ferritin (FtMt), encoded by the intronless FTMT gene on chromosome 5q in the human genome. FtMt is located in the mitochondrial matrix and expressed in cells with high metabolic activity, such as heart and brain. Mitochondrial ferritin is a H-type ferritin, suggesting that its role is not merely for iron storage, but possibly to control the mitochondrial LIP, required for various metabolic processes [53]. FtMt mRNA lacks the classical iron responsive element (IRE) stem-loop, and therefore does not seem to be translationally regulated by cellular iron levels unlike H- and L-ferritin. FtMt exhibits a N-terminal leader sequence for mitochondrial import. Once in mitochondria, the leader sequence is cleaved and FtMt peptides form the 24-mer ferritin shell structure to incorporate iron elements in a process similar to that of cytosolic H/L-ferritins [54]. The mature amino-acid sequence of FtMt shares 77% identity with H-ferritin and contains all residues of the ferroxidase center [55]. However, despite their similarities in ferroxidase centers, H-ferritin and FtMt display differences in their oxidation and hydrolysis chemistry. FtMt does not regenerate its ferroxidase activity after oxidation of the initial  $\text{Fe}^{2+}$

and has slower ferrooxidation and iron mineralization rates [56]. Therefore, FtMt is capable of controlling labile iron levels in mitochondria. Interestingly, FtMt has been found to protect cells from oxidative damage and erastin-induced ferroptosis in neuronal cells [57].

### **Serum ferritin**

Ferritin can also be secreted by cells [58, 59] and be found in the serum, where it comprises mostly of L-ferritin subunits [60]. L-ferritin is mainly involved in iron storage and mineralization. Thus, serum ferritin may carry iron, but not load labile iron that is present outside of the cell. Importantly, a substantial fraction of serum ferritin has been proposed to result from macrophages [59, 61]. Mammalian ferritins have no signal peptide for the classical endoplasmic reticulum-Golgi secretion pathway. Instead, a 13 amino-acid motif unique to ferritins has been identified on the BC-loop of both H- and L-subunits, which appears to be essential for ferritin secretion [58]. It is unknown if ferritin is secreted in a monomeric or multimeric form. It is also unknown if iron is bound to ferritin during the secretion process or if iron can be loaded onto ferritin in the serum, which poses the question to what extent serum ferritin levels are indicative of serum iron concentrations. These questions remain to be answered to better understand the role of serum ferritin as an iron carrier, delivery or storage system.

### **Ferritin in health and disease**

#### **Ferritin as a prognostic factor**

Most ferritin in the human body is found inside cells and a small amount is present in the serum, where it can carry iron [60]. Serum ferritin is low in iron content compared to its cellular form [62, 63], but it can still make a major contribution to cellular iron delivery [45]. Serum ferritin levels are often used to estimate iron content in the body, and it is commonly used as a diagnostic factor in blood tests. High serum ferritin levels can reflect hereditary and acquired iron-overload disorders such as hemochromatosis or transfusion therapy, whereas low ferritin levels can indicate iron-deficiency anemia [64]. There seems to be a positive correlation between disease progression and serum ferritin levels. Diagnostic values showed that serum ferritin is a very powerful indicator for the diagnosis of iron deficiency, being more powerful than red cells or transferrin iron saturation [64]. Transferrin iron saturation might indeed not be a very reliable measurement for body iron levels, given the fact that other cellular iron uptake pathways exist, such as CD44 and Lipocalin-2 [51, 65]. However, measurements of serum ferritin levels do not provide clues as to whether ferritin is in a monomeric or multimeric form, and in the latter case, if the ferritin shell is empty, fully loaded with iron or in an intermediate state. The amount of iron measured by serum ferritin can vary from one patient to another, taking all these factors into account [66]. Looking directly at the iron content of serum might be a better approximation of body iron levels. Ferritin levels can give additional information on iron storage, delivery and inflammation but it should not be considered as an absolute indicator of iron levels for diagnostic and prognostic purposes. Ferritin may also be a prognostic factor for certain types of cancers, and there have been reports in breast cancer [67, 68], ovarian cancer [69], pancreatic cancer [70] and advanced non-small-cell lung cancer [71] among others.

## Ferritin in inflammation

Serum ferritin has been described as a marker of acute and chronic inflammation and is elevated in a plethora of inflammatory conditions, including rheumatoid arthritis, systemic lupus erythematosus, chronic kidney disease, Coronavirus disease 2019 (COVID-19) caused by the virus SARS-CoV-2, acute infection, thyroiditis and others [72-77]. Interestingly, elevated ferritin levels have been detected in the synovial fluid and cells of rheumatoid arthritis patients [78], and ferritin levels have been reported to correlate with disease severity [79]. Ferritin has been documented to be a marker of inflammation and M1 macrophage activation [72]. Hyperferritinemia (serum ferritin  $\geq 500$  ng/mL) is a potential marker to discriminate a subset of hospitalized patients with confirmed influenza A infection, presenting high risk of developing poor outcome such as respiratory failure, admission to the intensive care unit or in-hospital mortality [80]. A significant rise in serum ferritin can indicate the activation of the monocyte-macrophage system, which is a crucial part of the inflammatory cytokine storm. Importantly, patients with COVID-19, which are in a severe state often die because of a resulting cytokine storm, constituting an excessive uncontrolled inflammatory response. Inflammation serum biomarkers, interleukin-6, coagulation indices and ferritin [74, 81] have been indicative of a potential inflammatory storm in the majority of patients with severe COVID-19. Importantly, diabetic COVID-19 patients present higher levels of inflammation biomarkers including high ferritin levels, linking cell metabolism and iron in this context [82]. However, the study does not document which type of diabetes is concerned. An important question is whether ferritin is a mediator or a consequence of inflammation. H-ferritin seems to modulate macrophage responses to immune stimuli and to play an important role in the protection against iron-induced oxidative stress [83]. Overexpression of H-ferritin in macrophages leads to their polarization towards M1 or M2 state depending on the cytokines present in their environment [84]. Moreover, in a rodent model of spinal cord injury (SCI), ferritin density was increased in the cytosol and lysosomes of macrophages in both injured (spinal cord) and non-injured (spleen) tissues of SCI as compared to naïve animals. This increase was accompanied by elevated serum ferritin levels in SCI rats, suggesting that SCI induces Toll-like receptor 4 activation, stimulating systemic iron sequestration, and thus promoting macrophage uptake of iron released by hemorrhage and cell death caused by SCI [85]. However, a number of previous studies have described both pro-inflammatory and immunosuppressive roles of ferritin, which may depend on the context and the various signaling pathways activated, but also on the role and localization of involved ferritin molecules, the H/L ratio in multimers and the potential role of monomeric ferritin in these inflammatory pathways. The function of ferritin as an iron provider or storage could be key and its regulation could explain the different observations.

In kidney disease, elevated ferritin levels have been documented to be associated with elevated mortality in the three regions studied (Europe, Japan and the United States), despite the different median ferritin levels across regions. However, its use as a biomarker is limited due to intravenous iron dosing, inflammation, anemia management strategies, or diet that may differ from one region to another [86]. Since dialysis patients often take iron supplements due to iron loss during the dialysis process, this opens the debate on safety of iron supplementation. This also raises the question to what extent ferritin and iron have implications in the etiology of this disease. Further studies are needed to shed light on this and to improve patient care.

## **Ferritin in cancer diagnosis**

Iron and ferritin levels are aberrant in many types of cancer [87]. Cancer cells often exhibit enhanced iron levels, either via an upregulation of cellular iron uptake and retention, or via decreased iron export. Often, this is concomitant with increased ferritin levels in cancer cells compared to normal tissues. Increased intracellular iron has diverse roles in cancer etiology, including metabolism, cancer growth and metastasis formation. Ferritin is upregulated in many cancer types and facilitates increased iron storage while limiting iron-dependent reactive oxygen species (ROS) production [88, 89]. Increased serum ferritin levels have been associated with poor prognosis in multiple types of cancers, including breast cancer [90, 91], colorectal cancer [92], liver cancer [93], lung cancer [94, 95], diffuse large B cell lymphoma [96], prostate cancer [97] and oral cancer [98] among others. Interestingly, serum iron levels are a better prognostic and diagnostic cancer marker than serum ferritin, suggesting that serum ferritin and iron levels are not necessarily correlated [99]. This could be due to different iron loading onto serum ferritin between patients. Cancer is often associated with anemia and the increased levels of ferritin could reflect intracellular iron accumulation in tumor cells, or cancer-related inflammation involving tumor-associated macrophages.

## **Ferritin and cellular plasticity in cancer**

The ability of cells to alter their phenotypes in response to environmental stimuli is known as cellular plasticity. In this process, chromatin states undergo changes that can be intimately tied to the hallmarks of cancer. Stress induced by the tumor microenvironment or therapeutic intervention can modify DNA and histone demethylases and reshape the chromatin landscape, potentially leading to plasticity and drug resistance [100]. In epithelial tumors, cancer stem cells (CSC), a subset exhibiting stem-like properties, undergo epithelial-to-mesenchymal transition (EMT) through which they lose their polarity and adhesion properties to gain a migratory and invasive phenotype and give rise to metastases, once migrated to distant sites in the body [101, 102]. EMT is modulated at different levels including epigenetic modifications, mitochondrial metabolism, transcriptional and translational controls, alternative splicing, protein stability and subcellular localization [102]. As CSC are a major contributor to drug-resistance, relapse and metastasis, and thus, targeting these cells represent a major challenge in cancer treatment.

Iron homeostasis is dysregulated in CSC and these cells display an increase in iron turnover and usually contain higher levels of ferritin [67, 69]. Recently, an alternative iron uptake mechanism has been discovered in mesenchymal tumor cells. Instead of taking up iron via the classical transferrin receptor pathway, cells undergoing EMT favor the alternative CD44-mediated pathway to internalize iron-bound hyaluronates [51] (Figure 3). Unlike transferrin receptor (TfR1) that is negatively regulated by excess iron, CD44 is transcriptionally upregulated by nuclear iron, thus allowing iron to be taken up by mesenchymal cells to a higher extent. The study further shows that nuclear iron operates as a metal catalyst for histone demethylation as a rate-limiting factor for epigenetic plasticity. In particular, the iron-dependent demethylase PHD finger protein 8 (PHF8), which removes the histone H3 Lysine 9 dimethyl (H3K9me2) repressive mark, plays an important role in governing the expression of mesenchymal genes, and thus, cell plasticity. This was associated with an increased level of nuclear ferritin in the mesenchymal state, which may be important for nuclear iron delivery and/or storage. Another study on breast cancer cells showed an upregulation of ferritin expression in cells with an aggressive mesenchymal phenotype, which was accompanied by an increase of nuclear H-ferritin levels and lower levels of labile iron



[91]. In glioblastoma initiating cells, H-ferritin was upregulated and constituted a critical factor for tumor cell survival [103]. Therefore, H-Ferritin may play a major role in tumor cell survival and plasticity. However, the mechanism by which iron is translocated into the nucleus and the precise role of nuclear ferritin remain to be elucidated. H-ferritin may mediate the translocation of iron into the nucleus and/or is required in the nucleus for iron storage. It may also provide labile iron via reduction of the ferritin iron core. All of this requires further studies to elucidate its precise role in epigenetic plasticity. Interestingly, in A549 lung cancer cells, transforming growth factor  $\beta$ 1-induced EMT enhances H-ferritin degradation by ferritinophagy and labile iron leakage from lysosomes, leading to ROS production, and thus to further autophagy during EMT [104]. Ferritin degradation in these cells leads to increased labile iron, which could provide an alternative mechanism to supply histone demethylases with iron, which merits further investigation. In ovarian cancer, H-Ferritin acted as a repressor of cancer proliferation and CSC propagation via the inhibition of EMT. It was suggested that this regulation was at least partly operated through a subset of miRNAs involved in cell migration and EMT gene expression [105]. Taken together, the link between ferritin, including H/L-chain composition, and the labile iron pool in different cell types and cell compartments should be investigated further.

As iron is upregulated in specific cancer cells, there are two main approaches to target them: deprive cancer cells of iron, or in the contrary, utilize excess iron of cancer cells to induce their death. Iron chelators such as deferoxamine (DFO) [51, 104] or DpdtC [106] can be used to inhibit EMT. Interestingly, using fluorescent labeling of a synthetic derivative of DFO that can undergo click chemistry [107], this molecule was predominantly found in the nucleus, arguing that DFO inhibits EMT via inhibition of iron-dependent histone demethylation [51]. In cancers associated with an upregulation of H-ferritin, its down-regulation by siRNA could be used to sensitize mesenchymal tumor cells to chemotherapy, as shown for breast cancer [91], or radiotherapy as shown for glioblastoma [103]. Interestingly, some small molecules can be used to sensitize CSC to ferroptosis. A synthetic derivative of salinomycin, called ironomycin, has been developed and showed potent and selective activity against breast CSC [67]. Thus, there is increased lysosomal iron sequestration, which induces oxidative stress, lysosomal membrane permeabilization and cell death by ferroptosis. Lysosomal membrane permeabilization could be either a cause or a result of cell death, and lipidomics experiments on the different compartments might yield more answers [108]. Lysosomal iron sequestration is a powerful strategy to selectively target cells with increased iron endocytosis [67, 109-114]. Artesunate, a drug used to treat malaria, can induce lysosomal degradation in an autophagy-independent manner, by increasing the cellular free iron level and thus oxidative stress and cell death. Besides, by interfering with the iron responsive elements/iron regulatory protein (IRE/IRP) signaling, artesunate can further increase cellular free iron [115, 116].

### **Ferritin glycosylation in diseases**

Ferritin can be found as glycosylated and non-glycosylated forms. Initially, it was proposed that *O*-glycosylation sites on H-ferritin may be involved in nuclear translocation [49, 117]. However, this mechanism remains poorly understood and requires further investigation. It has also been reported that a large portion of secreted L-ferritins are N-glycosylated. The ratio between glycosylated and non-glycosylated forms varies in diseases such as the adult onset Still's disease or rheumatoid arthritis [118]. In addition, elevated levels of serum ferritin and low levels of glycosylated ferritin were associated with both adult-onset Still's disease [119] and hemophagocytic lymphohistiocytosis [120]. As both are inflammatory diseases and low glycosylated ferritin levels have also been associated with

excessive macrophage activation [121], serum ferritin glycosylation may have a tight link with the immune system. Glycosylated ferritin is currently used as a biomarker for the previously mentioned diseases, but its regulation and effects are not yet understood and this requires mechanistic investigation. Another study found that human immunodeficiency virus 1 (HIV-1) patients treated with a highly active antiretroviral therapy (HAART) presented hyperglycosylated serum ferritin [122]. Since HIV targets immune white blood cells, this result would support the role of ferritin glycosylation in macrophage activation. Taken together, glycosylated ferritin has been found in a variety of diseases and how this is linked to iron homeostasis in these different settings needs to be investigated for potential novel therapeutic developments.

### **Implications of ferritin in other diseases**

There are genetic diseases that implicate ferritin (Figure 4). Hereditary ferritinopathy or neuroferritinopathy is a rare autosomal-dominant disease, characterized by an abnormal accumulation of iron and ferritin inclusion bodies in the brain [123]. It is caused by a mutation in the FTL gene, inducing a modification of the C-terminal region of L-ferritin, which causes disorder at the four-fold channels allowing iron leakage and enhanced formation of improperly coordinated iron. Thus, mutations in L-ferritin alter the functionality of the ferritin multimer and enhanced iron permeability [124]. In addition, the mutations cause a change in the overall conformation and stability of the ferritin multimer [125]. Since these disruptions of the ferritin multimer structures deeply impede its stability and function, this results in an impairment of iron homeostasis, which can lead to subsequent iron-induced oxidative damage. Neuroferritinopathy is characterized by the formation of ferritin inclusion bodies as cells overexpress ferritin in order to counter iron accumulation. However, ferritin protein aggregation may only be a secondary effect and not the cause of the pathology [124]. Another genetic mutation is known to be involved in ferritin-related diseases by causing hyperferritinemia. Human eukaryotic translation initiation factor 3 (eIF3) acts as a repressor of FTL mRNA translation, by binding to the 5'-UTR immediately adjacent to the IRE, and providing additional translational regulation independent of the IRE/IRP machinery. Single-nucleotide polymorphisms in the 5'-UTR of FTL gene disrupt the eIF3-mediated repression of FTL gene, thus causing and overexpression of ferritin. However, the potential role of eIF3 in iron level regulation remains to be clarified, especially in light of H/L-ferritin heteropolymers [126].

Dysregulated iron mechanism and abnormal ferritin levels have been detected in patients with neurological disorders [127] including Alzheimer's disease (AD) and Parkinson's disease (PD), and this has been linked to potential increased iron-induced oxidative damage in these disease settings [128]. Abnormal iron and ferritin levels and distributions have been found in diseased brains of AD patients [129]. Interestingly, a strong accumulation of ferritin was detected in neuritic plaques in the hippocampus of AD patients, almost exclusively associated with the microglia [130] and an increase of cortical iron and ferritin levels was also observed in amyloid plaques of a mouse model of AD [131]. It is not known if this ferritin accumulation is linked to the disease etiology or a consequence of the disease. Increased ferritin levels were also detected in the cerebrospinal fluid of AD patients, but not in patients with PD [132]. In addition, a study that imaged the localization of iron and ferritin in the hippocampus of an AD hippocampus found that ferritin was abundant in the cytoplasm and nucleus of oligodendrocytes and it was especially expressed in myelinated axons associated with oligodendrocyte processes [133]. The authors postulated that an increase of toxic brain ferrous ion may contribute to the production of free radicals that

induce cellular oxidative stress and myelin breakdown in AD patients. There have been reports implicating iron and ferritin in the early-onset degenerative disease Friedreich's Ataxia (FA), including elevated iron levels [134]. However, serum ferritin levels were reported to be normal in patients with FA, arguing that iron chelation therapy may not be a viable strategy [135]. In this disease, mitochondrial ferritin has been reported to limit oxidative damage regulating mitochondrial iron availability [136]. Interestingly, mitochondrial ferritin has been described to have a protective role in several neurodegenerative diseases, including AD [137].

Ferritin has also been implicated in autoimmune diseases [138]. For instance, adult onset Still's disease is a disorder which is characterized by arthritis and hyperferritinemia in the majority of cases [139]. Interestingly, these elevated ferritin levels are glycosylated in this disease [140]. Ferritin is also a marker for systemic lupus erythematosus, where it was also detected in the urine and cerebrospinal fluid with patients with meningitis [141]. Dysfunction of iron metabolism has also been described in patients with multiple sclerosis (MS), including elevated serum ferritin levels [142]. Interestingly, in a mouse model for MS, injections of apoferritin attenuated disease, suggesting that ferritin could play a role in iron sequestration in this disease [143], potentially acting as a defense mechanism against oxidative damage due to increased iron turnover. The normal distribution patterns of ferritin and transferrin is changed in brain tissues from MS patients. Ferritin might be involved in the demyelination associated with MS, or a consequence thereof [144]. Polymyositis and dermatomyositis are inflammatory autoimmune myopathies characterized by disorders of the connective tissue, and elevated serum ferritin levels were detected especially in elderly patients [145]. Idiopathic generalized seizure and atypical restless leg syndrome have been characterized by low L-ferritin levels [146]. Thus, ferritin polymers consist mainly of H-ferritin subunits, leading to increased iron incorporation into the resulting H-ferritin homopolymer. This results in reduced cellular iron availability.

### **Iron mobilization from ferritin and ferritinophagy**

Iron stored in cytosolic ferritin can be used by the same cell or exported from the cell. In the latter case, ferroportin (Fpn) can mediate iron extraction from ferritin, and ferritin subunits can subsequently be mono-ubiquitinated, which generates ferritin cage disassembly followed by degradation of the subunits by the proteasome [147]. Fpn regulates iron export together with the hepcidin peptide [148, 149]. Iron mobilization can occur from intact ferritin, and recent studies have shed light on these processes [150, 151]. It is still unclear to what extent these processes occur in cells, which warrants further investigations. Several reducing agents can cause the release of cations from intact ferritin nanocages, including ascorbate, flavin mononucleotide sodium dithionate and superoxide [150, 152-155]. Whereas reduced flavins can mobilize all the iron from ferritin, ascorbate and glutathione are less efficient and only reduce a part of the iron core. Interestingly, iron mobilization from ferritin by reduced flavins is favored *in vitro* only if all soluble oxygen is consumed, which is unlikely the case in a cellular environment. This puts into question, if these reactions are relevant in a cellular aerobic environment [150]. These reagents cause the reduction of ferritin iron to produce soluble  $\text{Fe}^{2+}$  cations, which exit the ferritin internal cavity through the eight three-fold channels to join the cellular LIP. The channels of the ferritin nanocage are too big for these reducing reagents to freely diffuse [156], suggesting that electron transfer is most likely how the ferritin iron core can be reduced by this mechanism [157, 158]. Another mechanism of iron release by ferritin was demonstrated using NADH as a reducing agent in the presence of dissolved molecular oxygen [159]. Since the rates of iron mobilization differ between the various reducing agents, it can be hypothesized that the subcellular environment and chemical

composition play a role in iron mobilization from ferritin, adding a layer of control over this process.

In human cells, a major mechanism of ferritin degradation and subsequent iron release takes place in autolysosomes. Autophagy is a natural mechanism by which cells recycle or remove dysfunctional proteins or organelles, by sequestering them in autophagosomes and delivering them to lysosomes for degradation. Nuclear coactivator 4 (NCOA4) plays the role of a selective cargo receptor directing ferritin to autolysosomes for degradation leading to iron release [160, 161] (Figure 3). The process of ferritin degradation by lysosomes through autophagy is called ferritinophagy. NCOA4 binds to ferritin complexes, and to autophagy-related protein 8 proteins that recruit cargo-receptor complexes into autophagosomes. This interaction leading to ferritinophagy was increased in iron-depleted conditions, experimentally stimulated by iron chelators. In the ferritin 24-mer, it has been determined by a mobility-shift assay that each H-ferritin subunit can bind one NCOA4 to form a complex with high stability that could not be dissociated by urea, NaCl or  $\beta$ -mercaptoethanol [43]. Remarkably, recent evidence showed that about eight molecules of NCOA4 can bind one ferritin nanocage [162]. Using an enzyme-linked immunosorbent assay, the binding was partially inhibited by  $\text{Fe}^{2+}$  but not by other divalent metal ions, which means that when labile iron is abundant, it prevents ferritin from degradation by interfering with NCOA4-mediated interaction with autophagosomes. In addition, NCOA4 binds selectively to H-ferritin and not L-ferritin, and can also bind to FtMt due to its 79% sequence homology to H-ferritin. The binding to L-ferritin may not be necessary as ferritin is usually in a heteropolymeric form and is already bound to NCOA4 via H-ferritin. The study further determined that the binding occurs through the NCOA4 (383-522) fragment and the R23A mutant H-ferritin could not bind to NCOA4. After degradation of iron-loaded ferritin in lysosomes, the ferrihydrite crystal dissolves when exposed to the acidic environment provided by lysosomes, and subsequently iron ions are released and reduced in the lysosomal fluid. These ions are transported back to the cytosol via divalent metal transporter 1 (DMT1) or natural resistance associated-macrophage protein 1 (Nramp1) depending on cell types, before being transferred to the blood for a use in another part of the organism, or used by the cell for endogenous iron-dependent processes [163]. Within the cell, iron is required by mitochondria to maintain their functions through a) iron-sulfur clusters [164] and heme-containing proteins, mainly present in the electron transport chain and b) redox homeostasis with other metals and metabolites. Iron deficiency alters mitochondrial respiration, the respiratory chain complex assembly and membrane potential. Under iron-deprived conditions, NCOA4-mediated ferritinophagy contributes to the maintenance of mitochondrial functions through iron release within the cell and supply to mitochondria [165].

Ferritinophagy and its regulation by NCOA4 has implications in health and disease [161]. NCOA4-mediated ferritinophagy may have implications in neurodegeneration [166], and NCOA4 has been detected in murine and rat brains [167]. However, detailed studies will be required to establish a clear link and clearer picture in this context.

Interestingly, NCOA4-mediated ferritinophagy has been reported to be required for erythropoiesis as its depletion in an *in vitro* model of erythroid differentiation impaired hemoglobinization and differentiation [168]. In a murine model it was also shown that NCOA4-mediated ferritinophagy is important to sustain erythropoiesis [169]. Initial studies on zebrafish documented that NCOA4 mRNA expression was upregulated at sites of erythropoiesis [170], and transcriptional analyses of erythroblasts showed that NCOA4 was highly upregulated in orthochromatic erythroblasts, where hemoglobin synthesis is highest [171].

## Ferroptosis

A novel form of regulated cell death, distinct from apoptosis and necrosis was coined in 2012 [7]. This iron-dependent cell death pathway, called ferroptosis, is characterized by the production of ROS from accumulated iron and by lipid peroxidation [8, 172]. Ferritinophagy can contribute to ferroptosis via NCOA4-mediated autophagic degradation of ferritin and subsequent release of labile iron in fibroblasts and cancer cells [173, 174]. Ferritinophagy plays an important role in cystine deprivation-induced ferroptosis in glioblastoma cells [175]. Recently, it was also demonstrated in glioblastoma cells, that increase of NCOA4-mediated autophagy increases vulnerability to ferroptosis. This was shown using the downregulation of coatamer protein complex subunit zeta 1 (COPZ1) in glioblastoma cells [176]. High levels of labile  $\text{Fe}^{2+}$  induce oxidative stress and therefore lipid peroxidation, leading to cell death (Figure 3). However, iron-independent ROS may also be involved in induction of autophagy mediated by erastin, a small-molecule ferroptosis inducer [177]. Ferroptosis is implicated in various human diseases, such as neurodegenerative diseases, ischemia/reperfusion injury, infectious diseases and importantly cancer (Figure 4). Ferroptosis is also involved in chronic obstructive pulmonary disease pathogenesis, mainly caused by tobacco smoking. Particulate matters contained in cigarette smoke, including iron, are deposited in smokers' lungs and alter iron homeostasis, resulting in oxidative stress and inflammation, subsequently leading to ferroptosis [178]. Another study provided evidence of iron dysregulation in AD and the possible implication of ferroptosis [179]. Interestingly, a recent study showed that iron stored in ferritin is reduced in the presence of AD peptide  $\beta$ -amyloid ( $\text{A}\beta$ ) [180], a peptide commonly found in AD patients. How this relates to disease etiology will need further investigation.

Persister cancer cells have often acquired a dependency on glutathione peroxidase 4 (GPX4), an enzyme that protects cells against lipid peroxidation, thus acting as a negative regulator of ferroptosis (Figure 3). Therefore, using GPX4 inhibitors such as RAS-selective lethal 3 (RSL3) [181] and ML210 [182] to trigger ferroptosis in these cells may be a promising strategy to selectively remove them and prevent cancer relapse. However, because of their poor pharmacokinetic properties, *in vivo* use of these two compounds is challenging [183]. A previously uncharacterized family of nitrile oxide masked electrophiles has recently been described as molecules specifically targeting GPX4 forming a covalent bond, potentially being promising compounds for drug development [184]. A complementary approach is to use natural molecules such as proteins and modify them to target cancer cells. A carrier-free nanodrug, called nanoparticle ferritin-bound erastin and rapamycin, has been developed to target GPX4 and induce ferroptosis [185]. Another approach is to target lysosomal iron [67, 109], creating ROS via the Fenton reaction, exploiting the fact that cancer stem cells show increased iron turnover [67, 69, 110, 186]. Salinomycin is a molecule in development and several analogues are being developed to induce ferroptosis in persister cancer cells [111]. Interestingly, a recent study also identified regulation of cellular iron export to be involved in ferroptosis resistance in breast cancer cells [187].

## Applications in bio-nanotechnology and therapeutics

Ferritin has been utilized as a versatile building block for various nanotechnology applications [188, 189] and we highlight some of these here. Ferritin is a very attractive particle in bionanotechnology due to its versatile properties and possible interactions with inorganic nanomaterials. In addition, the uniform size of ferritin multimers makes it an ideal material for ordered nanostructures and the protein can be modified either via genetic

engineering [190] or direct chemical modification [191]. This has created some very interesting composite biomaterials with diverse applications. For instance, a stable and biodegradable nanoparticle made out of ferritin was prepared to be able to reduce Cr(IV) into non-toxic Cr(III) [192]. Another report described the development of a phosphate removal system from water based on this protein [193]. Carbon nanotubes have also been prepared that include ferritin as a catalyst [194]. An interesting application for ferritin is the use as a contrasting agent for magnetic resonance imaging (MRI) in mice. To this end, ferritin has been exogenously introduced via transfection of H-ferritin [195] or via viral vectors [196]. Ferritin was also cloned into rabies virus vectors, which can pass the blood–brain barrier [197]. Intravenously injected cationized ferritin was also followed by MRI and the particles were found neither nephrotoxic nor hepatotoxic and did not increase leukocyte counts in healthy rats [198]. Tissue-specific vectors encoding for ferritin were also used to visualize neurons mice using MRI [199]. However, one has to consider that introducing ferritin, in particular if loaded with iron, can trigger changes in iron levels and distribution, which can have an effect on the MRI images. Ferritin subunits can be genetically modified to add functions at the nanocage surface without altering the formation of the cage-like structure. The multivalent protein surface of ferritin offers useful functionalities, which can be used to conjugate ligands such as antibodies, as was shown for autoantibodies of Type I diabetes in the sera of patients [200]. Importantly, material can be obtained in high yield using bacterial cultivation, which is relatively inexpensive. Since the N-terminus is outside of the protein shell, it is easily amenable to modifications, and it was reported that the C-terminus can be modified without altering the capability of monomer folding or multimer assembly [201]. Taken together, this opens plenty of avenues for therapeutic and analytical biomedical applications.

Due to its size, hollow cavity, non-toxic biodegradability and the fact that it can be taken up into cells directly, ferritin can be developed into a drug delivery system (Figure 4). For instance, protein nanocages are being developed to potentially deliver encapsulated proteins into tumor cells [202]. Besides, by playing with pH-dependent disassembly/reassembly properties of ferritin, it is possible to trap compounds inside the multimer. Ferritin also presents assets for medical imaging thanks to the iron contained in its cavity. A ferritin-based drug delivery system has been developed where subunits were modified to have a cage with CGKRR peptides on its surface, targeting specifically the tumor angiogenic blood vessels and tumor cells [203]. The modified ferritin was loaded with a near infrared-absorbing organometallic complex, named ‘556-Ph’. This new drug delivery system showed promising results on MDA-MB-435 tumor cells and xenograft tumors for imaging-guided photodynamic therapy and photothermal therapy. Another study extended the C-terminal ends of the human ferritin subunits with a peptide that targets tumor cell receptors, and loaded this ferritin with ~800 arsenates and ~1100 iron atoms to make it toxic for breast cancer cells. Once arsenate-loaded ferritin was taken up by cancer cells, it was directed to lysosomes for degradation and release of arsenate, which subsequently led to cell death [204]. However, the cytotoxicity of this approach remains to be tested on non-cancerous cells. Ferritin can also be artificially modified to contain in its core a magnetic oxide (maghemite or magnetite). Such modified ferritin is called magnetoferritin. Due to its stability, biocompatibility and magnetic properties, magnetoferritin has been widely exploited for imaging and biomedical applications [205] (Figure 4).

Ferritin-based radionuclide nanoparticles have also been developed for cancer radio imaging and radioimmunotherapy [206] [207]. However, this strategy has proven problematic due to weak specificity and competition with other elements. The increased iron metabolism of some cancer cells [67, 69] may provide the means to using ferritin receptor mediated mechanisms in order to transfer drugs to these cells. To this end ferritin was conjugated to

lipid nanoparticles bearing the widely-used anti-cancer drug 5-fluorouracil [208]. This increased uptake in MDA-MB-468 cells [208], which were also shown to have plastic properties on the epithelial-to-mesenchymal spectrum, with an increased iron load in the mesenchymal state [51]. Interestingly, these ferritin-based nanoparticles caused a reduction in tumor growth in MDA-MB-468 tumor-bearing Balb/c mice [208]. Magnetic nanoparticles have also been developed to detect ferritin accumulation in AD [209].

## Conclusions

Recent studies have brought new insights into ferritin structure and its mechanism of self-assembly [14, 15, 17], which allow a better understanding of the multiple roles of ferritin in iron homeostasis, as well as ferritin selectivity for iron ions over other metals. Different organs and tissues have distinct distributions of H-and L-ferritin, affecting their biology. Ferritin multimers rich in L-ferritin tend to have more of a storage function, whereas multimers rich in H-ferritin tend to have functions beyond iron storage, including regulation of the LIP or the supply of labile iron. Distinct distributions of different forms of ferritin within cellular compartments also have profound biological consequences. Whereas L-ferritin is predominant in the cytosol, H-ferritin is enriched in the cell nucleus and mitochondrial ferritin has properties closely related to H-ferritin.

The regular multimeric structures that ferritin can adapt, as well as the possibility to modify the protein at various sites, makes it a useful molecule for applications in nanobiotechnology. These properties and the fact that ferritin can be taken up into cells directly also makes it an interesting vector for drug delivery.

Ferritin has been studied extensively for its role in iron storage and protection from oxidative stress. Iron homeostasis is deregulated in many diseases and serum ferritin is used as a biomarker of body iron levels and/or inflammation. This is particularly interesting, as there is evidence that correlates serum ferritin levels with ferritin leakage from damaged cells [210]. Since serum ferritin levels are modified in many diseases in an unspecific manner, it should be combined with other more specific biomarkers for diagnosis and prognosis, considering variations among populations (type of treatment, age, gender, social background, geographical regions, etc.), but also looking at ferritin structure (monomer or multimer, H/L ratio, iron loading). It might be also pertinent to look at mitochondrial ferritin levels and LIP in cells to see whether they are also deregulated, especially considering that mitochondrial iron metabolism can play key roles in tumorigenesis and cancer cell plasticity [51, 211, 212]. Interestingly, other metals such as copper also play a role in this process [213], and how these metals interplay with one another especially in the mitochondria will be an interesting line of further research.

Ferritin is involved in cancer progression through EMT giving rise to CSC, and a set of small molecules have been developed to target this subset of cancer cells by exploiting their inherent iron upregulation. Whereas the bulk of the literature has reported an upregulation of ferritin in these settings, there have been reports of opposite effects of ferritin on cancer proliferation. There might be tissue-specific or cancer-specific effects at play. In addition, ferritinophagy seems to play a role in these reports, hinting towards another mechanism to provide labile iron. There has been interesting recent work on how ferritin provides labile iron other than through NCOA4-mediated ferritinophagy including reductive mobilization [150, 151]. It will be important to study, which of these processes occur in what cellular compartment, in order to understand iron-dependent biological processes better, for instance iron-catalyzed histone demethylation occurring in the nucleus during cellular plasticity in cancer cells [51].

In addition, more detailed studies on the functions of ferritin and its cellular localization in different cell types and diseases may give valuable information about the control of iron pools in different settings, including labile iron. We now know that ferritin is also involved in macrophage activation but the underlying mechanisms remain to be elucidated. In addition, studies have shown either pro-inflammatory or immunosuppressive roles of H-ferritin, posing an obvious question, whether ferritin is a mere iron storage protein or displays other functions, such as controlling the LIP. This has widespread implications in diseases that have inflammatory responses, including respiratory infections such as influenza or COVID-19. This could be regulated by a careful balance in ferritin subunits and heteropolymer composition as well as differential ferritin localization within the cell. In addition, specific post-translational modifications of ferritin in specific cellular settings may play differential roles with respect to iron regulation. Ferritin monomers might also play a role in specific cell compartments by interacting with some molecules or by playing a role in ferritin translocation. The same reasoning can be made for cancer, as ferritinophagy can help cell growth and in other cases trigger ferroptosis. This obviously also puts into question the practice of assessing iron load in patients only through serum ferritin concentrations. Without knowledge of polymer composition and iron loading, these reading can be misleading. To correctly understand the roles of ferritin in iron homeostasis and diseases, efforts should be made to identify the link between the roles, localizations, structures and compositions of ferritin as well as its interactions with other molecules and effects on labile iron levels.

#### Data availability

All figures are available upon request. No new primary data are included in the article.

#### References

1. Editorial: Iron as Soul of Life on Earth Revisited: From Chemical Reaction, Ferroptosis to Therapeutics. *Free Rad Biol Med* 2019; **133**.
2. Ng SS, Kavanagh KL, McDonough MA *et al*: Crystal structures of histone demethylase JMJD2A reveal basis for substrate specificity. *Nature* 2007; **448**:87-91.
3. Jian N, Dowle M, Horniblow RD *et al*: Morphology of the ferritin iron core by aberration corrected scanning transmission electron microscopy. *Nanotechnology* 2016; **27**:46LT02.
4. Melino G, Stefanini S, Chiancone E *et al*: Stoichiometry of iron oxidation by apoferritin. *FEBS Lett* 1978; **86**:136-138.
5. Arosio P, Elia L, Poli M: Ferritin, cellular iron storage and regulation. *IUBMB Life* 2017; **69**:414-422.
6. Theil EC: Ferritin: the protein nanocage and iron biomineral in health and in disease. *Inorg Chem* 2013; **52**:12223-12233.
7. Dixon SJ, Lemberg KM, Lamprecht MR *et al*: Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012; **149**:1060-1072.
8. Conrad M, Pratt DA: The chemical basis of ferroptosis. *Nat Chem Biol* 2019; **15**:1137-1147.



9. Torti SV, Manz DH, Paul BT *et al*: Iron and Cancer. *Annu Rev Nutr* 2018; **38**:97-125.
10. Lundin D, Poole AM, Sjoberg BM *et al*: Use of structural phylogenetic networks for classification of the ferritin-like superfamily. *J Biol Chem* 2012; **287**:20565-20575.
11. He D, Hughes S, Vanden-Hehir S *et al*: Structural characterization of encapsulated ferritin provides insight into iron storage in bacterial nanocompartments. *Elife* 2016; **5**:e18972.
12. Ross J, Lambert T, Piergentili C *et al*: Mass spectrometry reveals the assembly pathway of encapsulated ferritins and highlights a dynamic ferroxidase interface. *Chem Commun* 2020; **56**:3417-3420.
13. Arosio P, Ingrassia R, Cavadini P: Ferritins: a family of molecules for iron storage, antioxidation and more. *Biochim Biophys Acta* 2009; **1790**:589-599.
14. Sato D, Ohtomo H, Yamada Y *et al*: Ferritin Assembly Revisited: A Time-Resolved Small-Angle X-ray Scattering Study. *Biochemistry* 2016; **55**:287-293.
15. Sato D, Ikeguchi M: Mechanisms of ferritin assembly studied by time-resolved small-angle X-ray scattering. *Biophys Rev* 2019; **11**:449-455.
16. Carmona F, Poli M, Bertuzzi M *et al*: Study of ferritin self-assembly and heteropolymer formation by the use of Fluorescence Resonance Energy Transfer (FRET) technology. *Biochim Biophys Acta Gen Subj* 2017; **1861**:522-532.
17. Houben L, Weissman H, Wolf SG *et al*: A mechanism of ferritin crystallization revealed by cryo-STEM tomography. *Nature* 2020; **579**:540-543.
18. Treffry A, Zhao Z, Quail MA *et al*: Dinuclear center of ferritin: studies of iron binding and oxidation show differences in the two iron sites. *Biochemistry* 1997; **36**:432-441.
19. Melman A, Bou-Abdallah F: Iron mineralization and core dissociation in mammalian homopolymeric H-ferritin: Current understanding and future perspectives. *Biochim Biophys Acta Gen Sub* 2020; **1864**:129700.
20. Mehlenbacher M, Poli M, Arosio P *et al*: Iron Oxidation and Core Formation in Recombinant Heteropolymeric Human Ferritins. *Biochemistry* 2017; **56**:3900-3912.
21. Bou-Abdallah F, Zhao G, Biasiotto G *et al*: Facilitated diffusion of iron(II) and dioxygen substrates into human H-chain ferritin. A fluorescence and absorbance study employing the ferroxidase center substitution Y34W. *J Am Chem Soc* 2008; **130**:17801-17811.
22. Zhao G, Bou-Abdallah F, Arosio P *et al*: Multiple pathways for mineral core formation in mammalian apoferritin. The role of hydrogen peroxide. *Biochemistry* 2003; **42**:3142-3150.
23. Svobodova H, Kosnac D, Tanila H *et al*: Iron-oxide minerals in the human tissues. *Biometals* 2020; **33**:1-13.

24. Cowley JM, Janney DE, Gerkin RC *et al*: The structure of ferritin cores determined by electron nanodiffraction. *J Struct Biol* 2000; **131**:210-216.
25. Honarmand Ebrahimi K, Bill E, Hagedoorn PL *et al*: The catalytic center of ferritin regulates iron storage via Fe(II)-Fe(III) displacement. *Nat Chem Biol* 2012; **8**:941-948.
26. Chen P, De Meulenaere E, Deheyn DD *et al*: Iron redox pathway revealed in ferritin via electron transfer analysis. *Sci Rep* 2020; **10**:4033.
27. Laghaei R, Evans DG, Coalson RD: Metal binding sites of human H-chain ferritin and iron transport mechanism to the ferroxidase sites: a molecular dynamics simulation study. *Proteins* 2013; **81**:1042-1050.
28. Behera RK, Theil EC: Moving Fe<sup>2+</sup> from ferritin ion channels to catalytic OH centers depends on conserved protein cage carboxylates. *Proc Natl Acad Sci USA* 2014; **111**:7925-7930.
29. Chandramouli B, Del Galdo S, Mancini G *et al*: Mechanistic insights into metal ions transit through threefold ferritin channel. *Biochim Biophys Acta Gen Subj* 2019; **1863**:472-480.
30. Behera RK, Torres R, Tosha T *et al*: Fe(2+) substrate transport through ferritin protein cage ion channels influences enzyme activity and biomineralization. *J Biol Inorg Chem* 2015; **20**:957-969.
31. Philpott CC, Ryu MS, Frey A *et al*: Cytosolic iron chaperones: Proteins delivering iron cofactors in the cytosol of mammalian cells. *J Biol Chem* 2017; **292**:12764-12771.
32. Shi H, Bencze KZ, Stemmler TL *et al*: A cytosolic iron chaperone that delivers iron to ferritin. *Science* 2008; **320**:1207-1210.
33. Ryu MS, Duck KA, Philpott CC: Ferritin iron regulators, PCBP1 and NCOA4, respond to cellular iron status in developing red cells. *Blood Cells Mol Dis* 2018; **69**:75-81.
34. Patel SJ, Frey AG, Palenchar DJ *et al*: A PCBP1-BolA2 chaperone complex delivers iron for cytosolic [2Fe-2S] cluster assembly. *Nat Chem Biol* 2019; **15**:872-881.
35. Lavoie DJ, Marcus DM, Otsuka S *et al*: Characterization of ferritin from human placenta. Implications for analysis of tissue specificity and microheterogeneity of ferritins. *Biochim Biophys Acta* 1979; **579**:359-366.
36. Thompson K, Menzies S, Muckenthaler M *et al*: Mouse brains deficient in H-ferritin have normal iron concentration but a protein profile of iron deficiency and increased evidence of oxidative stress. *J Neurosci Res* 2003; **71**:46-63.
37. Linder MC, Goode CA, Gonzalez R *et al*: Heart tissue contains small and large aggregates of ferritin subunits. *Arch Biochem Biophys* 1989; **273**:34-41.

38. Ahmad S, Moriconi F, Naz N *et al*: Ferritin L and Ferritin H are differentially located within hepatic and extra hepatic organs under physiological and acute phase conditions. *Int J Clin Exp Pathol* 2013; **6**:622-629.
39. Matsuno T, Mori M, Awai M: Distribution of ferritin and hemosiderin in the liver, spleen and bone marrow of normal, phlebotomized and iron overloaded rats. *Acta Med Okayama* 1985; **39**:347-360.
40. Bevilacqua MA, Faniello MC, Russo T *et al*: P/CAF/p300 complex binds the promoter for the heavy subunit of ferritin and contributes to its tissue-specific expression. *Biochem J* 1998; **335**:521-525.
41. Cairo G, Rappocciolo E, Tacchini L *et al*: Expression of the genes for the ferritin H and L subunits in rat liver and heart. Evidence for tissue-specific regulations at pre- and post-translational levels. *Biochem J*; 1991; **275 ( Pt 3)**:813-816.
42. Wilkinson Jt, Di X, Schonig K *et al*: Tissue-specific expression of ferritin H regulates cellular iron homeostasis in vivo. *Biochem J* 2006; **395**:501-507.
43. Gryzik M, Srivastava A, Longhi G *et al*: Expression and characterization of the ferritin binding domain of Nuclear Receptor Coactivator-4 (NCOA4). *Biochim Biophys Acta Gen Subj* 2017; **1861**:2710-2716.
44. Fuhrmann DC, Mondorf A, Beifuss J *et al*: Hypoxia inhibits ferritinophagy, increases mitochondrial ferritin, and protects from ferroptosis. *Redox Biol* 2020; **36**:101670.
45. Li JY, Paragas N, Ned RM *et al*: Scara5 is a ferritin receptor mediating non-transferrin iron delivery. *Dev Cell* 2009; **16**:35-46.
46. Li L, Fang CJ, Ryan JC *et al*: Binding and uptake of H-ferritin are mediated by human transferrin receptor-1. *Proc Natl Acad Sci USA* 2010; **107**:3505-3510.
47. Cai CX, Birk DE, Linsenmayer TF: Nuclear ferritin protects DNA from UV damage in corneal epithelial cells. *Mol Biol Cell* 1998; **9**:1037-1051.
48. Surguladze N, Thompson KM, Beard JL *et al*: Interactions and reactions of ferritin with DNA. *J Biol Chem* 2004; **279**:14694-14702.
49. Surguladze N, Patton S, Cozzi A *et al*: Characterization of nuclear ferritin and mechanism of translocation. *Biochem J* 2005; **388**:731-740.
50. Kubilus JK, Beazley KE, Talbot CJ *et al*: Nuclear ferritin mediated regulation of JNK signaling in corneal epithelial cells. *Exp Eye Res* 2016; **145**:337-340.
51. Müller S, Sindikubwabo F, Cañeque T *et al*: CD44 regulates epigenetic plasticity by mediating iron endocytosis. *Nat Chem* 2020.
52. Millholland JM, Fitch JM, Cai CX *et al*: Ferritoid, a tissue-specific nuclear transport protein for ferritin in corneal epithelial cells. *J Biol Chem* 2003; **278**:23963-23970.
53. Drysdale J, Arosio P, Invernizzi R *et al*: Mitochondrial ferritin: a new player in iron metabolism. *Blood Cells Mol Dis* 2002; **29**:376-383.

54. Langlois d'Estaintot B, Santambrogio P, Granier T *et al*: Crystal structure and biochemical properties of the human mitochondrial ferritin and its mutant Ser144Ala. *J Mol Biol* 2004; **340**:277-293.
55. Corsi B, Cozzi A, Arosio P *et al*: Human mitochondrial ferritin expressed in HeLa cells incorporates iron and affects cellular iron metabolism. *J Biol Chem* 2002; **277**:22430-22437.
56. Bou-Abdallah F, Santambrogio P, Levi S *et al*: Unique iron binding and oxidation properties of human mitochondrial ferritin: a comparative analysis with Human H-chain ferritin. *J Mol Biol* 2005; **347**:543-554.
57. Wang YQ, Chang SY, Wu Q *et al*: The Protective Role of Mitochondrial Ferritin on Erastin-Induced Ferroptosis. *Front Aging Neurosci* 2016; **8**:1-9.
58. Truman-Rosentsvit M, Berenbaum D, Spektor L *et al*: Ferritin is secreted via 2 distinct nonclassical vesicular pathways. *Blood* 2018; **131**:342-352.
59. Cohen LA, Gutierrez L, Weiss A *et al*: Serum ferritin is derived primarily from macrophages through a nonclassical secretory pathway. *Blood* 2010; **116**:1574-1584.
60. Wang W, Knovich MA, Coffman LG *et al*: Serum ferritin: Past, present and future. *Biochim Biophys Acta* 2010; **1800**:760-769.
61. Yuan XM, Li W, Baird SK *et al*: Secretion of ferritin by iron-laden macrophages and influence of lipoproteins. *Free Radic Res* 2004; **38**:1133-1142.
62. Worwood M, Dawkins S, Wagstaff M *et al*: The purification and properties of ferritin from human serum. *Biochem J* 1976; **157**:97-103.
63. Arosio P, Yokota M, Drysdale JW: Characterization of serum ferritin in iron overload: possible identity to natural apoferritin. *Br J Haematol* 1977; **36**:199-207.
64. Guyatt GH, Oxman AD, Ali M *et al*: Laboratory diagnosis of iron-deficiency anemia: an overview. *J Gen Intern Med* 1992; **7**:145-153.
65. Flo TH, Smith KD, Sato S *et al*: Lipocalin 2 mediates an innate immune response to bacterial infection by sequestering iron. *Nature* 2004; **432**:917-921.
66. Hallberg L, Bengtsson C, Lapidus L *et al*: Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. *Br J Haematol* 1993; **85**:787-798.
67. Mai TT, Hamai A, Hienzsch A *et al*: Salinomycin kills cancer stem cells by sequestering iron in lysosomes. *Nat Chem* 2017; **9**:1025-1033.
68. Weinstein RE, Bond BH, Silberberg BK *et al*: Tissue ferritin concentration and prognosis in carcinoma of the breast. *Breast Cancer Res Treat* 1989; **14**:349-353.
69. Basuli D, Tesfay L, Deng Z *et al*: Iron addiction: a novel therapeutic target in ovarian cancer. *Oncogene* 2017; **36**:4089-4099.

70. Wang SL, Cao S, Wu R *et al*: Serum ferritin predicted prognosis in patients with locally advanced pancreatic cancer. *Future Oncol* 2015; **11**:2905-2910.
71. Wu Z, Dai Y, Chen LA: The Prediction Of Epidermal Growth Factor Receptor Mutation And Prognosis Of EGFR Tyrosine Kinase Inhibitor By Serum Ferritin In Advanced NSCLC. *Cancer Manag Res* 2019; **11**:8835-8843.
72. Ruscitti P, Di Benedetto P, Berardicurti O *et al*: Pro-inflammatory properties of H-ferritin on human macrophages, ex vivo and in vitro observations. *Sci Rep* 2020; **10**:12232.
73. Cheng L, Li H, Li L *et al*: Ferritin in the coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. *J Clin Lab Anal* 2020; **34**:e23618.
74. Dahan S, Segal G, Katz I *et al*: Ferritin as a Marker of Severity in COVID-19 Patients: A Fatal Correlation. *Isr Med Assoc J* 2020; **8**:429-434.
75. Seyhan S, Pamuk ON, Pamuk GE *et al*: The correlation between ferritin level and acute phase parameters in rheumatoid arthritis and systemic lupus erythematosus. *Eur J Rheumatol* 2014; **1**:92-95.
76. Ueda N, Takasawa K: Impact of Inflammation on Ferritin, Hcpidin and the Management of Iron Deficiency Anemia in Chronic Kidney Disease. *Nutrients* 2018; **10**.
77. Sakata S, Nagai K, Maekawa H *et al*: Serum ferritin concentration in subacute thyroiditis. *Metabolism* 1991; **40**:683-688.
78. Blake DR, Bacon PA, Eastham EJ *et al*: Synovial fluid ferritin in rheumatoid arthritis. *Br Med J* 1980; **281**:715-716.
79. Yildirim K, Karatay S, Melikoglu MA *et al*: Associations between acute phase reactant levels and disease activity score (DAS28) in patients with rheumatoid arthritis. *Ann Clin Lab Sci* 2004; **34**:423-426.
80. Lalueza A, Ayuso B, Arrieta E *et al*: Elevation of serum ferritin levels for predicting a poor outcome in hospitalized patients with influenza infection. *Clin Microbiol Infect* 2020.
81. Lin Z, Long F, Yang Y *et al*: Serum ferritin as an independent risk factor for severity in COVID-19 patients. *J Infect* 2020.
82. Guo W, Li M, Dong Y *et al*: Diabetes is a risk factor for the progression and prognosis of COVID-19. *Diabetes Metab Res Rev* 2020:e3319.
83. Mesquita G, Silva T, Gomes AC *et al*: H-Ferritin is essential for macrophages' capacity to store or detoxify exogenously added iron. *Sci Rep* 2020; **10**:3061.
84. Bolisetty S, Zarjou A, Hull TD *et al*: Macrophage and epithelial cell H-ferritin expression regulates renal inflammation. *Kidney Int* 2015; **88**:95-108.

85. Blissett AR, Deng B, Wei P *et al*: Sub-cellular In-situ Characterization of Ferritin(iron) in a Rodent Model of Spinal Cord Injury. *Sci Rep* 2018; **8**:3567.
86. Karaboyas A, Morgenstern H, Pisoni RL *et al*: Association between serum ferritin and mortality: findings from the USA, Japan and European Dialysis Outcomes and Practice Patterns Study. *Nephrol Dial Transplant* 2018; **33**:2234-2244.
87. Torti SV, Torti FM: Iron and ferritin in inflammation and cancer. *Adv Inorg Biochem* 1994; **10**:119-137.
88. Salatino A, Aversa I, Battaglia AM *et al*: H-Ferritin Affects Cisplatin-Induced Cytotoxicity in Ovarian Cancer Cells through the Modulation of ROS. *Oxid Med; Cell Longev* 2019; **2019**:3461251.
89. Buranrat B, Connor JR: Cytoprotective effects of ferritin on doxorubicin-induced breast cancer cell death. *Oncol Rep* 2015; **34**:2790-2796.
90. Chekhun SV, Lukyanova NY, Shvets YV *et al*: Significance of ferritin expression in formation of malignant phenotype of human breast cancer cells. *Exp Oncol* 2014; **36**:179-183.
91. Shpyleva SI, Tryndyak VP, Kovalchuk O *et al*: Role of ferritin alterations in human breast cancer cells. *Breast Cancer Res Treat* 2011; **126**:63-71.
92. Feng Z, Chen JW, Feng JH *et al*: The association between serum ferritin with colorectal cancer. *Int J Clin Exp Med* 2015; **8**:22293-22299.
93. Wu SJ, Zhang ZZ, Cheng NS *et al*: Preoperative serum ferritin is an independent prognostic factor for liver cancer after hepatectomy. *Surg Oncol* 2019; **29**:159-167.
94. Lee S, Jeon H, Shim B: Prognostic Value of Ferritin-to-Hemoglobin Ratio in Patients with Advanced Non-Small-Cell Lung Cancer. *J Cancer* 2019; **10**:1717-1725.
95. Ferrigno D, Buccheri G: Serum ferritin levels in lung cancer patients. *Eur J Cancer* 1992; **28**:241.
96. Kim DJ, Kim T, Jeong JY *et al*: Poor prognostic impact of high serum ferritin levels in patients with a lower risk of diffuse large B cell lymphoma. *Int J Hematol* 2020; **111**:559-566.
97. Wang X, An P, Zeng J *et al*: Serum ferritin in combination with prostate-specific antigen improves predictive accuracy for prostate cancer. *Oncotarget* 2017; **8**:17862-17872.
98. Baharvand M, Manifar S, Akkafan R *et al*: Serum levels of ferritin, copper, and zinc in patients with oral cancer. *Biomed J* 2014; **37**:331-336.
99. Chua AC, Knuiman MW, Trinder D *et al*: Higher concentrations of serum iron and transferrin saturation but not serum ferritin are associated with cancer outcomes. *Am J Clin Nutr* 2016; **104**:736-742.

100. Flavahan WA, Gaskell E, Bernstein BE: Epigenetic plasticity and the hallmarks of cancer. *Science* 2017; **357**.
101. Müller S, Cañeque T, Acevedo V *et al*: Targeting cancer stem cells with small molecules. *Israel Journal of Chemistry* 2017; **57**:239-250.
102. Nieto MA, Huang RY, Jackson RA *et al*: EMT: 2016. *Cell* 2016; **166**:21-45.
103. Ravi V, Madhankumar AB, Abraham T *et al*: Liposomal delivery of ferritin heavy chain 1 (FTH1) siRNA in patient xenograft derived glioblastoma initiating cells suggests different sensitivities to radiation and distinct survival mechanisms. *PLoS One* 2019; **14**:e0221952.
104. Sioutas A, Vainikka LK, Kentson M *et al*: Oxidant-induced autophagy and ferritin degradation contribute to epithelial-mesenchymal transition through lysosomal iron. *J Inflamm Res* 2017; **10**:29-39.
105. Lobello N, Biamonte F, Pisanu ME *et al*: Ferritin heavy chain is a negative regulator of ovarian cancer stem cell expansion and epithelial to mesenchymal transition. *Oncotarget* 2016; **7**:62019-62033.
106. Feng J, Li C, Xu R *et al*: DpdtC-Induced EMT Inhibition in MGC-803 Cells Was Partly through Ferritinophagy-Mediated ROS/p53 Pathway. *Oxid Med Cell Longev* 2020; **2020**:9762390.
107. Cañeque T, Müller S, Rodriguez R: Visualizing biologically active small molecules in cells using click chemistry. *Nat Rev Chem* 2018; **2**:202-215.
108. El Hout M, Dos Santos L, Hamai A *et al*: A promising new approach to cancer therapy: Targeting iron metabolism in cancer stem cells. *Semin Cancer Biol* 2018; **53**:125-138.
109. Turcu AL, Versini A, Khene N *et al*: DMT1 Inhibitors Kill Cancer Stem Cells by Blocking Lysosomal Iron Translocation. *Chem Eur J* 2020; **26**:7369-7373.
110. Hamai A, Cañeque T, Müller S *et al*: An iron hand over cancer stem cells. *Autophagy* 2017; **13**:1465-1466.
111. Versini A, Colombeau L, Hienzsch A *et al*: Salinomycin Derivatives Kill Breast Cancer Stem Cells by Lysosomal Iron Targeting. *Eur J Chemistry* 2020; **26**:7416-7424.
112. Laraia L, Garivet G, Foley DJ *et al*: Image-Based Morphological Profiling Identifies a Lysosomotropic, Iron-Sequestering Autophagy Inhibitor. *Angew Chem Int Ed* 2020; **59**:5721-5729.
113. Versini A, Saier L, Sindikubwabo F *et al*: Chemical biology of salinomycin. *Tetrahedron* 2018; **74**:5585-5614.
114. Cañeque T, Müller S, Lafon A *et al*: Reprogramming the chemical reactivity of iron in cancer stem cells. *Comptes Rendus Chimie* 2018; **21**:704-708.

115. Chen GQ, Benthani FA, Wu J *et al*: Artemisinin compounds sensitize cancer cells to ferroptosis by regulating iron homeostasis. *Cell Death Differ* 2020; **27**:242-254.
116. Yang ND, Tan SH, Ng S *et al*: Artesunate induces cell death in human cancer cells via enhancing lysosomal function and lysosomal degradation of ferritin. *J Biol Chem* 2014; **289**:33425-33441.
117. Alkhateeb AA, Connor JR: Nuclear ferritin: A new role for ferritin in cell biology. *Biochim Biophys Acta* 2010; **1800**:793-797.
118. Ghosh S, Hevi S, Chuck SL: Regulated secretion of glycosylated human ferritin from hepatocytes. *Blood* 2004; **103**:2369-2376.
119. Wang Z, Wang Y, Wang J *et al*: Early diagnostic value of low percentage of glycosylated ferritin in secondary hemophagocytic lymphohistiocytosis. *Int J Hematol* 2009; **90**:501-505.
120. Nabergoj M, Marinova M, Binotto G *et al*: Diagnostic and prognostic value of low percentage of glycosylated ferritin in acquired hemophagocytic lymphohistiocytosis: A single-center study. *Int J Lab Hematol* 2017; **39**:620-624.
121. Lambotte O, Cacoub P, Costedoat N *et al*: High ferritin and low glycosylated ferritin may also be a marker of excessive macrophage activation. *J Rheumatol* 2003; **30**:1027-1028.
122. Manfredi R, Calza L, Chiodo F: Long-term statin use does not act on the temporal trend of CD4 cell count in patients on virologically effective HAART. *AIDS* 2006; **20**:455-457.
123. Muhoberac BB, Vidal R: Iron, Ferritin, Hereditary Ferritinopathy, and Neurodegeneration. *Front Neurosci* 2019; **13**:1195.
124. McNally JR, Mehlenbacher MR, Luscieti S *et al*: Mutant L-chain ferritins that cause neuroferritinopathy alter ferritin functionality and iron permeability. *Metallomics* 2019; **11**:1635-1647.
125. Kuwata T, Okada Y, Yamamoto T *et al*: Structure, Function, Folding, and Aggregation of a Neuroferritinopathy-Related Ferritin Variant. *Biochemistry* 2019; **58**:2318-2325.
126. Pulos-Holmes MC, Srole DN, Juarez MG *et al*: Repression of ferritin light chain translation by human eIF3. *Elife* 2019; **8**.
127. Finazzi D, Arosio P: Biology of ferritin in mammals: an update on iron storage, oxidative damage and neurodegeneration. *Arch Toxicol* 2014; **88**:1787-1802.
128. Jellinger K, Paulus W, Grundke-Iqbal I *et al*: Brain iron and ferritin in Parkinson's and Alzheimer's diseases. *J Neural Transm Park Dis Dement Sect* 1990; **2**:327-340.
129. Connor JR, Menzies SL, St Martin SM *et al*: A histochemical study of iron, transferrin, and ferritin in Alzheimer's diseased brains. *J Neurosci Res* 1992; **31**:75-83.



130. Grundke-Iqbal I, Fleming J, Tung YC *et al*: Ferritin is a component of the neuritic (senile) plaque in Alzheimer dementia. *Acta Neuropathol* 1990; **81**:105-110.
131. Svobodova H, Kosnac D, Balazsiova Z *et al*: Elevated age-related cortical iron, ferritin and amyloid plaques in APP(swe)/PS1(deltaE9) transgenic mouse model of Alzheimer's disease. *Physiol Res* 2019; **68**:S445-S451.
132. Kuiper MA, Mulder C, van Kamp GJ *et al*: Cerebrospinal fluid ferritin levels of patients with Parkinson's disease, Alzheimer's disease, and multiple system atrophy. *J Neural Transm Park Dis Dement Sect* 1994; **7**:109-114.
133. Quintana C, Bellefqih S, Laval JY *et al*: Study of the localization of iron, ferritin, and hemosiderin in Alzheimer's disease hippocampus by analytical microscopy at the subcellular level. *J Struct Biol* 2006; **153**:42-54.
134. Llorens JV, Soriano S, Calap-Quintana P *et al*: The Role of Iron in Friedreich's Ataxia: Insights From Studies in Human Tissues and Cellular and Animal Models. *Front Neurosci* 2019; **13**:75.
135. Wilson RB, Lynch DR, Fischbeck KH: Normal serum iron and ferritin concentrations in patients with Friedreich's ataxia. *Ann Neurol* 1998; **44**:132-134.
136. Campanella A, Rovelli E, Santambrogio P *et al*: Mitochondrial ferritin limits oxidative damage regulating mitochondrial iron availability: hypothesis for a protective role in Friedreich ataxia. *Hum Mol Genet* 2009; **18**:1-11.
137. Yang H, Yang M, Guan H *et al*: Mitochondrial ferritin in neurodegenerative diseases. *Neurosci Res* 2013; **77**:1-7.
138. Zandman-Goddard G, Shoenfeld Y: Ferritin in autoimmune diseases. *Autoimmun Rev* 2007; **6**:457-463.
139. Uppal SS, Al-Mutairi M, Hayat S *et al*: Ten years of clinical experience with adult onset Still's disease: is the outcome improving? *Clin Rheumatol* 2007; **26**:1055-1060.
140. Van Reeth C, Le Moel G, Lasne Y *et al*: Serum ferritin and isoferritins are tools for diagnosis of active adult Still's disease. *J Rheumatol* 1994; **21**:890-895.
141. Nishiya K, Hashimoto K: Elevation of serum ferritin levels as a marker for active systemic lupus erythematosus. *Clin Exp Rheumatol* 1997; **15**:39-44.
142. Sfagos C, Makis AC, Chaidos A *et al*: Serum ferritin, transferrin and soluble transferrin receptor levels in multiple sclerosis patients. *Mult Scler* 2005; **11**:272-275.
143. LeVine SM, Maiti S, Emerson MR *et al*: Apoferritin attenuates experimental allergic encephalomyelitis in SJL mice. *Dev Neurosci* 2002; **24**:177-183.
144. Hulet SW, Powers S, Connor JR: Distribution of transferrin and ferritin binding in normal and multiple sclerotic human brains. *J Neurol Sci* 1999; **165**:48-55.
145. Marie I, Hatron PY, Levesque H *et al*: Influence of age on characteristics of polymyositis and dermatomyositis in adults. *Medicine* 1999; **78**:139-147.

146. Cozzi A, Santambrogio P, Privitera D *et al*: Human L-ferritin deficiency is characterized by idiopathic generalized seizures and atypical restless leg syndrome. *J Exp Med* 2013; **210**:1779-1791.
147. De Domenico I, Vaughn MB, Li L *et al*: Ferroportin-mediated mobilization of ferritin iron precedes ferritin degradation by the proteasome. *EMBO J* 2006; **25**:5396-5404.
148. Donovan A, Brownlie A, Zhou Y *et al*: Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature* 2000; **403**:776-781.
149. Nemeth E, Tuttle MS, Powelson J *et al*: Heparin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; **306**:2090-2093.
150. Bou-Abdallah F, Paliakkara JJ, Melman G *et al*: Reductive Mobilization of Iron from Intact Ferritin: Mechanisms and Physiological Implication. *Pharmaceuticals* 2018; **11**:120.
151. Theil EC, Behera RK, Tosha T: Ferritins for Chemistry and for Life. *Coord Chem Rev* 2013; **257**:579-586.
152. Sirivech S, Frieden E, Osaki S: The release of iron from horse spleen ferritin by reduced flavins. *Biochem J* 1974; **143**:311-315.
153. Boyer RF, Grabill TW, Petrovich RM: Reductive release of ferritin iron: a kinetic assay. *Anal Biochem* 1988; **174**:17-22.
154. Sakurai K, Nabeyama A, Fujimoto Y: Ascorbate-mediated iron release from ferritin in the presence of alloxan. *Biometals* 2006; **19**:323-333.
155. Bolann BJ, Ulvik RJ: Release of iron from ferritin by xanthine oxidase. Role of the superoxide radical. *Biochem J* 1987; **243**:55-59.
156. Johnson LE, Wilkinson T, Arosio P *et al*: Effect of chaotropes on the kinetics of iron release from ferritin by flavin nucleotides. *Biochim Biophys Acta Gen Subj* 2017; **1861**:3257-3262.
157. Bradley JM, Svistunenko DA, Lawson TL *et al*: Three Aromatic Residues are Required for Electron Transfer during Iron Mineralization in Bacterioferritin. *Angew Chem Int Ed* 2015; **127**:14976-14980.
158. Bradley JM, Svistunenko DA, Moore GR *et al*: Tyr25, Tyr58 and Trp133 of Escherichia coli bacterioferritin transfer electrons between iron in the central cavity and the ferroxidase centre. *Metallomics* 2017; **9**:1421-1428.
159. Koochana PK, Mohanty A, Das S *et al*: Releasing iron from ferritin protein nanocage by reductive method: The role of electron transfer mediator. *Biochim Biophys Acta Gen Subj* 2018; **1862**:1190-1198.
160. Mancias JD, Wang X, Gygi SP *et al*: Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature* 2014; **509**:105-109.

161. Santana-Codina N, Mancias JD: The Role of NCOA4-Mediated Ferritinophagy in Health and Disease. *Pharmaceuticals* 2018; **11**.
162. Srivastava AK, Flint N, Kreckel H *et al*: Thermodynamic and Kinetic Studies of the Interaction of Nuclear Receptor Coactivator-4 (NCOA4) with Human Ferritin. *Biochemistry* 2020; **59**:2707-2717.
163. La A, Nguyen T, Tran K *et al*: Mobilization of iron from ferritin: new steps and details. *Metallomics* 2018; **10**:154-168.
164. Paul BT, Tesfay L, Winkler CR *et al*: Sideroflexin 4 affects Fe-S cluster biogenesis, iron metabolism, mitochondrial respiration and heme biosynthetic enzymes. *Sci Rep* 2019; **9**:19634.
165. Fujimaki M, Furuya N, Saiki S *et al*: Iron Supply via NCOA4-Mediated Ferritin Degradation Maintains Mitochondrial Functions. *Mol Cell Biol* 2019; **39**.
166. Quiles Del Rey M, Mancias JD: NCOA4-Mediated Ferritinophagy: A Potential Link to Neurodegeneration. *Front Neurosci* 2019; **13**:238.
167. Kollara A, Brown TJ: Variable expression of nuclear receptor coactivator 4 (NcoA4) during mouse embryonic development. *J Histochem Cytochem* 2010; **58**:595-609.
168. Mancias JD, Pontano Vaites L, Nissim S *et al*: Ferritinophagy via NCOA4 is required for erythropoiesis and is regulated by iron dependent HERC2-mediated proteolysis. *Elife* 2015; **4**.
169. Nai A, Lidonnici MR, Federico G *et al*: NCOA4-mediated ferritinophagy in macrophages is crucial to sustain erythropoiesis in mice. *Haematologica* 2020.
170. Weber GJ, Choe SE, Dooley KA *et al*: Mutant-specific gene programs in the zebrafish. *Blood* 2005; **106**:521-530.
171. An X, Schulz VP, Li J *et al*: Global transcriptome analyses of human and murine terminal erythroid differentiation. *Blood* 2014; **123**:3466-3477.
172. Perez MA, Magtanong L, Dixon SJ *et al*: Dietary Lipids Induce Ferroptosis in *Caenorhabditis elegans* and Human Cancer Cells. *Dev Cell* 2020; **54**:447-454 e444.
173. Hou W, Xie Y, Song X *et al*: Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* 2016; **12**:1425-1428.
174. Gao M, Monian P, Pan Q *et al*: Ferroptosis is an autophagic cell death process. *Cell Res* 2016; **26**:1021-1032.
175. Hayashima K, Kimura I, Katoh H: Role of ferritinophagy in cystine deprivation-induced cell death in glioblastoma cells. *Biochem Biophys Res Commun* 2021; **539**:56-63.
176. Zhang Y, Kong Y, Ma Y *et al*: Loss of COPZ1 induces NCOA4 mediated autophagy and ferroptosis in glioblastoma cell lines. *Oncogene* 2021.

177. Park E, Chung SW: ROS-mediated autophagy increases intracellular iron levels and ferroptosis by ferritin and transferrin receptor regulation. *Cell Death Dis* 2019; **10**:822.
178. Yoshida M, Minagawa S, Araya J *et al*: Involvement of cigarette smoke-induced epithelial cell ferroptosis in COPD pathogenesis. *Nat Commun* 2019; **10**:1-13.
179. Ashraf A, Jeandriens J, Parkes HG *et al*: Iron dyshomeostasis, lipid peroxidation and perturbed expression of cystine/glutamate antiporter in Alzheimer's disease: Evidence of ferroptosis. *Redox Biol* 2020; **32**:101494.
180. Everett J, Brooks J, Lermyte F *et al*: Iron stored in ferritin is chemically reduced in the presence of aggregating Abeta(1-42). *Sci Rep* 2020; **10**:10332.
181. Yang WS, Stockwell BR: Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. *Chem Biol* 2008; **15**:234-245.
182. Weiwer M, Bittker JA, Lewis TA *et al*: Development of small-molecule probes that selectively kill cells induced to express mutant RAS. *Bioorg Med Chem Lett* 2012; **22**:1822-1826.
183. Hangauer MJ, Viswanathan VS, Ryan MJ *et al*: Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* 2017; **551**:247-250.
184. Eaton JK, Furst L, Ruberto RA *et al*: Selective covalent targeting of GPX4 using masked nitrile-oxide electrophiles. *Nat Chem Biol* 2020; **16**:497-506.
185. Li Y, Wang X, Yan J *et al*: Nanoparticle ferritin-bound erastin and rapamycin: a nanodrug combining autophagy and ferroptosis for anticancer therapy. *Biomater Sci* 2019; **7**:3779-3787.
186. Torti SV, Torti FM: Iron: The cancer connection. *Mol Aspects Med* 2020:100860.
187. Brown CW, Amante JJ, Chhoy P *et al*: Prominin2 Drives Ferroptosis Resistance by Stimulating Iron Export. *Dev Cell* 2019; **51**:575-586 e574.
188. Jutz G, van Rijn P, Santos Miranda B *et al*: Ferritin: a versatile building block for bionanotechnology. *Chem Rev* 2015; **115**:1653-1701.
189. He D, Marles-Wright J: Ferritin family proteins and their use in bionanotechnology. *N Biotechnol* 2015; **32**:651-657.
190. Kramer RM, Li C, Carter DC *et al*: Engineered protein cages for nanomaterial synthesis. *J Am Chem Soc* 2004; **126**:13282-13286.
191. Perriman AW, Mann S: Liquid proteins--a new frontier for biomolecule-based nanoscience. *ACS Nano* 2011; **5**:6085-6091.
192. Kim I, Hosein H-A, Strongin DR *et al*: Photochemical Reactivity of Ferritin for Cr(VI) Reduction. *Chem Mater* 2002; **14**:4874-4879.

193. Jacobs JF, Hasan MN, Paik KH *et al*: Development of a bionanotechnological phosphate removal system with thermostable ferritin. *Biotechnol Bioeng* 2010; **105**:918-923.
194. Bonard JM, Chauvin P, Klinke C: Monodisperse Multiwall Carbon Nanotubes Obtained with Ferritin as Catalyst. *Nano Lett* 2002; **2**:665–667.
195. Klem MT, Mosolf J, Young M *et al*: Photochemical mineralization of europium, titanium, and iron oxyhydroxide nanoparticles in the ferritin protein cage. *Inorg Chem* 2008; **47**:2237-2239.
196. Vande Velde G, Rangarajan JR, Toelen J *et al*: Evaluation of the specificity and sensitivity of ferritin as an MRI reporter gene in the mouse brain using lentiviral and adeno-associated viral vectors. *Gene Ther* 2011; **18**:594-605.
197. Liu L, Gong K, Ming P *et al*: Reconstruction and expression of the MRI-contrast protein, ferritin, with recombinant rabies vectors. *Biotechnol Lett* 2010; **32**:743-748.
198. Beeman SC, Georges JF, Bennett KM: Toxicity, biodistribution, and ex vivo MRI detection of intravenously injected cationized ferritin. *Magn Reson Med* 2013; **69**:853-861.
199. Khodanovich MY, Akulov AE, Anan'ina TV *et al*: Tissue-Specific Ferritin- and GFP-Based Genetic Vectors Visualize Neurons by MRI in the Intact and Post-Ischemic Rat Brain. *Int J Mol Sci* 2020; **21**.
200. Lee SH, Lee H, Park JS *et al*: A novel approach to ultrasensitive diagnosis using supramolecular protein nanoparticles. *FASEB J* 2007; **21**:1324-1334.
201. Luzzago A, Cesareni G: Isolation of point mutations that affect the folding of the H chain of human ferritin in E.coli. *EMBO J* 1989; **8**:569-576.
202. Palombarini F, Di Fabio E, Boffi A *et al*: Ferritin Nanocages for Protein Delivery to Tumor Cells. *Molecules* 2020; **25**.
203. Zhang J, Zeng Y, Su M *et al*: Multifunctional Ferritin Nanoparticles as Theranostics for Imaging-Guided Tumor Phototherapy. *J Biomed Nanotechnol* 2019; **15**:1546-1555.
204. Cioloboc D, Kurtz DM, Jr.: Targeted cancer cell delivery of arsenate as a reductively activated prodrug. *J Biol Inorg Chem* 2020; **25**:441-449.
205. Xue L, Deng D, Sun J: Magnetoferritin: Process, Prospects, and Their Biomedical Applications. *Int J Mol Sci* 2019; **20**.
206. Wu H, Engelhard MH, Wang J *et al*: Synthesis of lutetium phosphate–apoferritin core–shell nanoparticles for potential applications in radioimmunoimaging and radioimmunotherapy of cancers. *J Mater Chem* 2008; **18**:1779–1783.
207. Wu H, Wang J, Wang Z *et al*: Apoferritin-templated yttrium phosphate nanoparticle conjugates for radioimmunotherapy of cancers. *J Nanosci Nanotechnol* 2008; **8**:2316-2322.

208. Jain SK, Chaurasiya A, Gupta Y *et al*: Development and characterization of 5-FU bearing ferritin appended solid lipid nanoparticles for tumour targeting. *J Microencapsul* 2008; **25**:289-297.
209. Fernandez T, Martinez-Serrano A, Cusso L *et al*: Functionalization and Characterization of Magnetic Nanoparticles for the Detection of Ferritin Accumulation in Alzheimer's Disease. *ACS Chem Neurosci* 2018; **9**:912-924.
210. Kell DB, Pretorius E: Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics* 2014; **6**:748-773.
211. Li C, Zhang Y, Cheng X *et al*: PINK1 and PARK2 Suppress Pancreatic Tumorigenesis through Control of Mitochondrial Iron-Mediated Immunometabolism. *Dev Cell* 2018; **46**:441-455 e448.
212. Gentric G, Kieffer Y, Mieulet V *et al*: PML-Regulated Mitochondrial Metabolism Enhances Chemosensitivity in Human Ovarian Cancers. *Cell Metab* 2019; **29**:156-173 e110.
213. Müller S, Versini A, Sindikubwabo F *et al*: Metformin reveals a mitochondrial copper addiction of mesenchymal cancer cells. *PLoS One* 2018; **13**:e0206764.

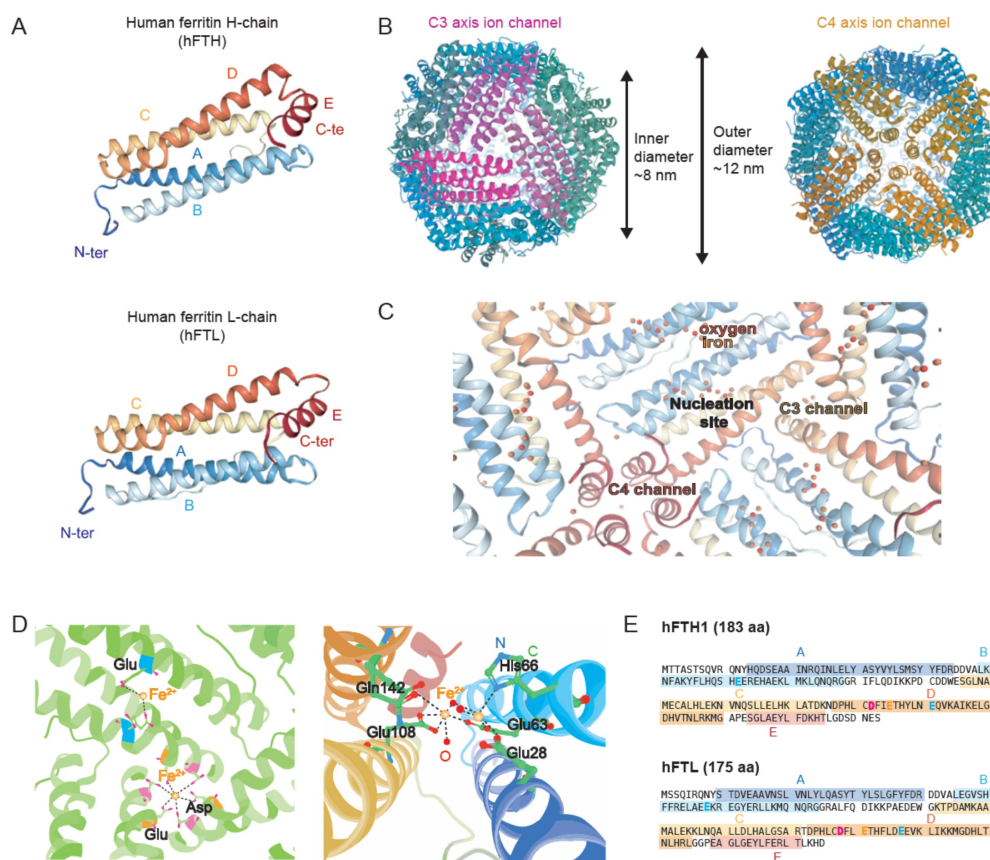
ORIGINAL UNEDITED MANUSCRIPT

## **Acknowledgments**

The R.R. research group is funded by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No [647973]), the Fondation Charles Defforey-Institut de France and Ligue Contre le Cancer (Equipe Labellisée). R.R. is supported by (centre national de la recherche scientifique) CNRS and S.M. by institut national de la santé et de la recherche médicale (INSERM).

ORIGINAL UNEDITED MANUSCRIPT

Figure 1



**Figure 1. Structure of ferritin subunits and the ferritin nanocage.**

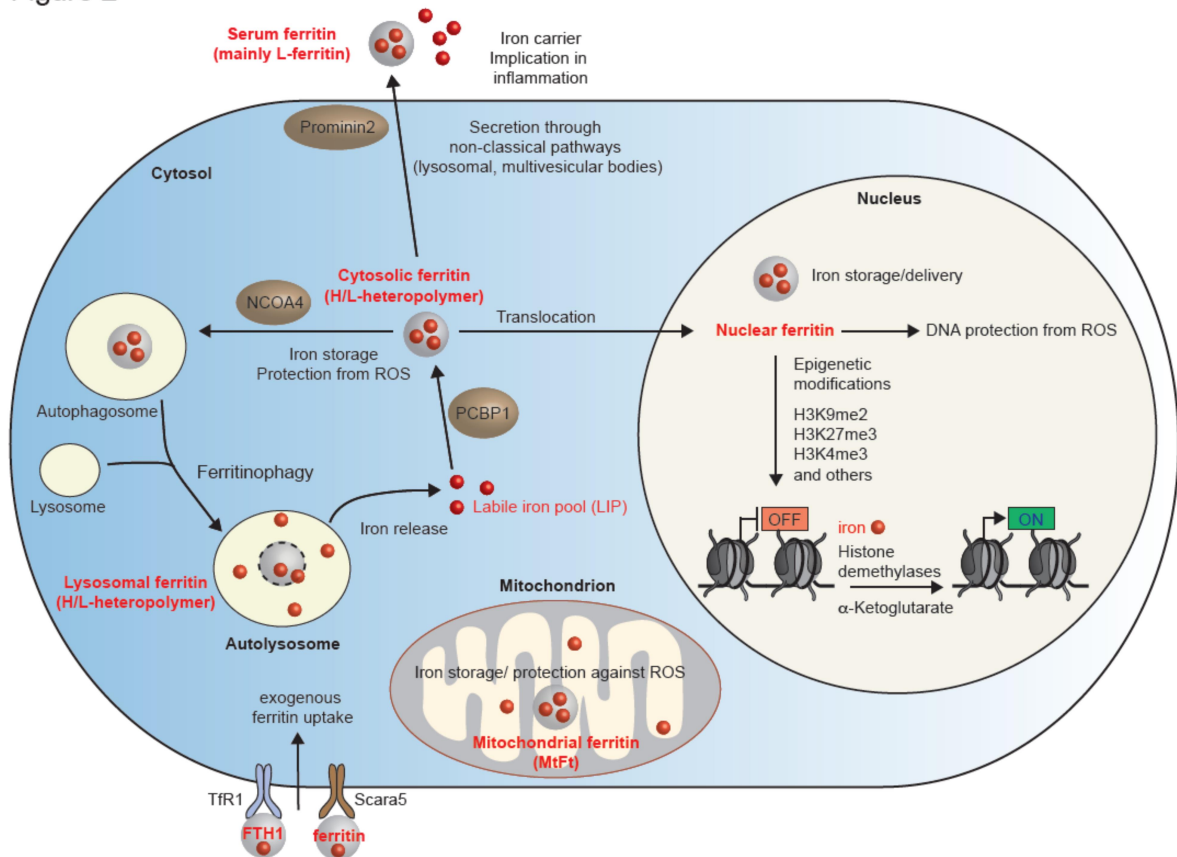
(A) Structures of human H-ferritin (PDB ID: 4ZJK) and L-ferritin (PDB ID: 6TSF). Despite ~55% of sequence homology between H- and L-ferritin, the 3D structures are similar. (B) Structures of the Ferritin 24-mer nanocage with C3 and C4 axes that constitute ions channels. (PDB ID: 2FG8). (C) Iron and oxygen elements inside the ferritin nanocage. Iron ions travel through threefold and fourfold channels. Iron and oxygen atoms accumulate at the nucleation site. (PDB ID: 6TSF). (D) Left panel: Fe<sup>2+</sup> binding sites in human H-ferritin (modified from PDB, ID: 3AJ0). Asp residues (pink) and Glu residues (orange) are located in the C3 channel. Glu residues (blue) permit the transit between C3 channel exit and ferroxidase site. The structure and binding sites are similar in L-ferritin. Right panel: Fe<sup>2+</sup> binding sites in the



ferroxidase center of H-ferritin (modified from PDB, ID: 4YKH). (E) H-ferritin and L-ferritin amino acids sequences with  $\text{Fe}^{2+}$  binding sites indicated with colors (from UniProtKB - P02792 (FRIL\_HUMAN) and P02794 (FRIH\_HUMAN)). Highlighted sequences and colored letters correspond to the A-E helices.

ORIGINAL UNEDITED MANUSCRIPT

Figure 2

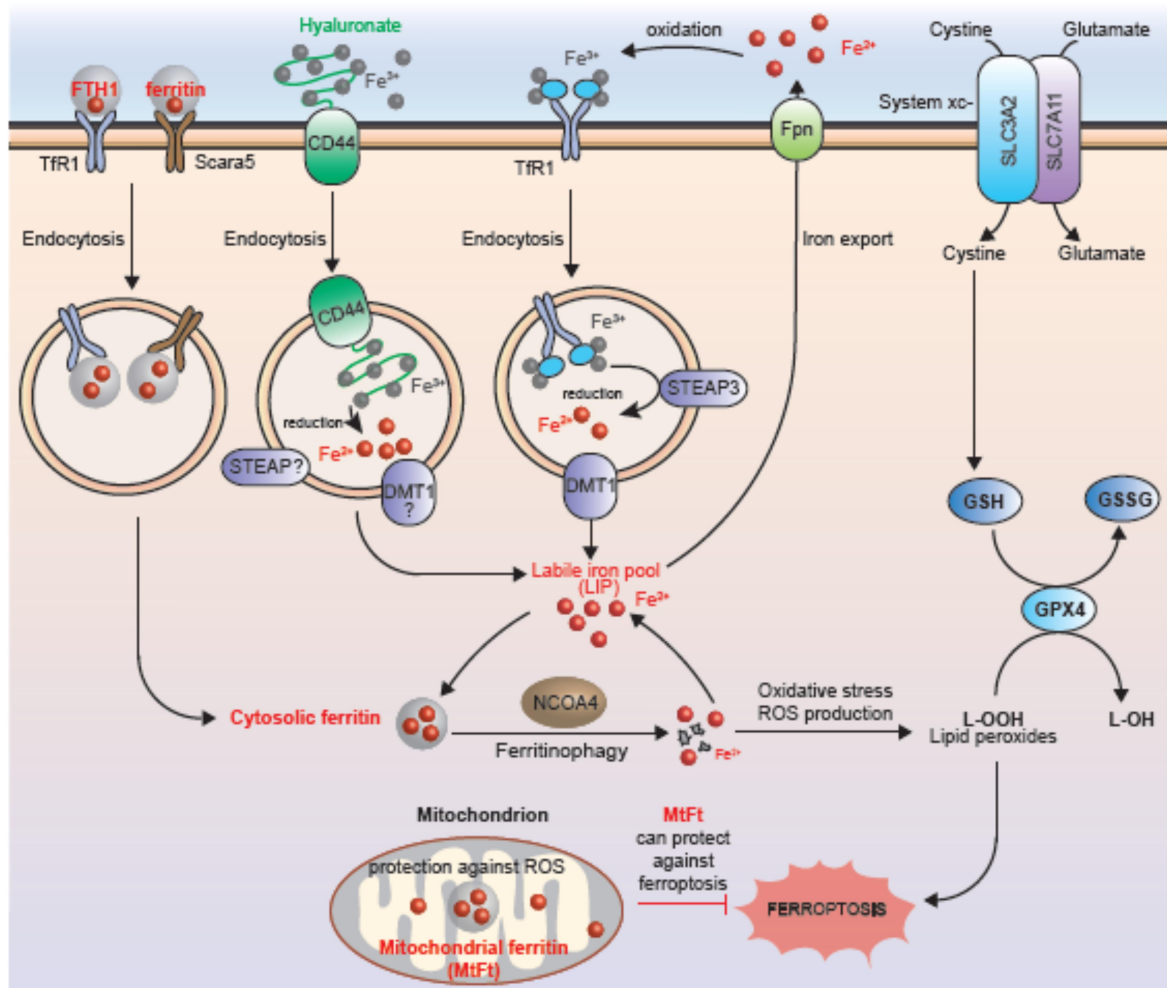


**Figure 2. Localizations and functions of ferritin in the cell.**

Scheme depicting ferritin localization and composition in the cell (written in red). The various functions of ferritin are indicated. Exogenous ferritin can be internalized via the membrane receptors TfR1 and scavenger receptor class A member 5 (Scara5) and excreted via a pathway dependent on the protein Prominin2. Excretion of ferritin is via non-classical pathways. Ferritin can be found in different cellular compartments, including the cytosol, the mitochondria and the nucleus, and can act as an iron storage facility. Ferritin translocates to the nucleus with an unknown mechanism. Within the nucleus ferritin can protect DNA from oxidative damage, provide an iron reservoir and potentially iron for processes such as histone and DNA demethylation. Iron is a central player controlling epigenetic plasticity. Mitochondrial ferritin can protect cells against ROS and thus ferroptosis. PCBP1 is a chaperone that delivers iron to ferritin. Ferritin degradation is mediated by NCOA4, which is a cargo receptor bringing ferritin to autophagosomes. Degradation of the ferritin multimer in autolysosomes liberates iron that will become part of the labile iron pool.

ORIGINAL PREPRINT

Figure 3

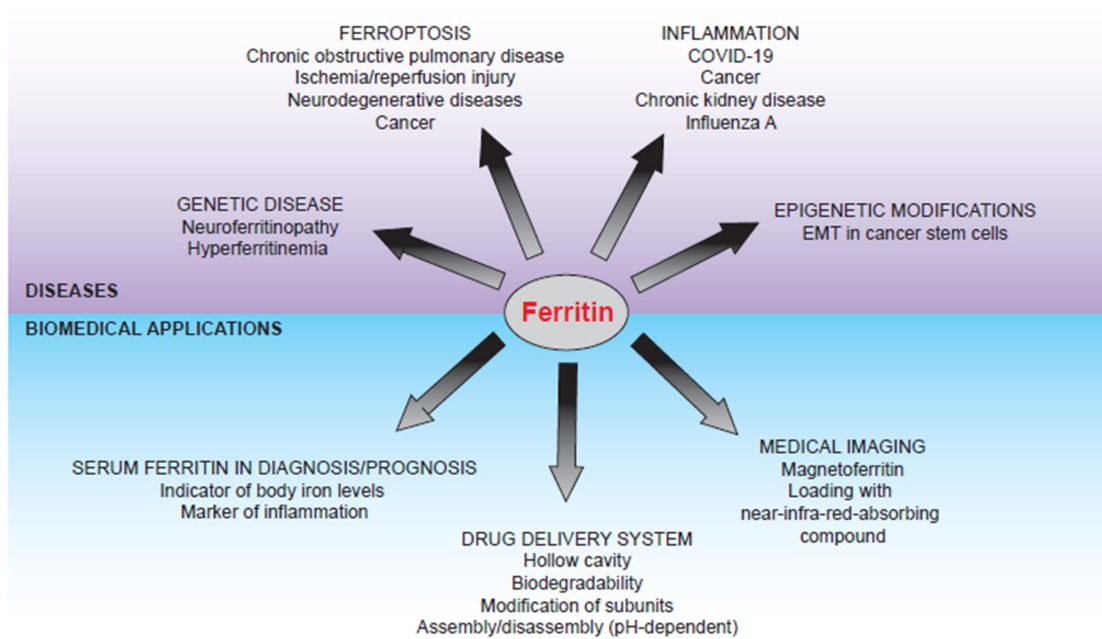


**Figure 3. Implications of cellular ferritin in ferroptosis.**

Schematics of cellular processes implicating ferritin as a central player in iron homeostasis. Ferric iron can be taken up by transferrin receptor (TfR1) or by CD44-hyaluronates, and is then reduced into ferrous iron before being released into the cytosol. STEAP3 is the reductase responsible for the reduction of ferric iron and DMT1 is the protein responsible for ferrous iron translocation to the cytosol. How these processes are mediated in CD44-mediated iron endocytosis needs further investigation. Exogenous ferritin can also be taken up directly by TfR1 or the receptor Scara5. Free ferrous iron can be stored inside ferritin multimers or exported via ferroportin (Fpn). Endocytosis and ferritin degradation are the main contributors to the labile iron pool. Labile iron can generate oxidative stress and production of lipid peroxides, subsequently leading to ferroptotic cell death. The accumulation of lipid peroxides is prevented by glutathione peroxidase 4 (GPX4). SGH = glutathione, GSSH = glutathione disulfide. MtFt can protect cells against ferroptosis.

ORIGINAL PAPER

Figure 4



**Figure 4. Ferritin in health and disease.**

Schematics depicting a list of non-exhaustive the involvement of ferritin in disease settings and biomedical applications. The upper section (purple) indicates implications of ferritin in various diseases. For this part, ferritin is involved in genetic diseases based on ferritin alterations, ferroptotic cell death, epigenetic modifications such as methylation and inflammation via its implications in iron homeostasis. The lower section (blue) shows the different uses of ferritin in biomedical applications. Three major axes constitute ferritin in diagnostics, drug delivery systems and medical imaging.