## **Supporting Information**

Routes of iron entry into, and exit from, the catalytic ferroxidase sites of the prokaryotic ferritin *Syn*Ftn

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			3	TR		В			
SynFtn	:	SMNPDLLSAIQQHISIERYASVTYLAMSIWCAEREL	AGFYQFFDGEA	KDEQSHAV	VHFTQYLIAF	SQSNDLQTLDAPF	QNWDSL	:	99
HumanH	:	NYHQDSEAAINRQINLELYASYVYLSMSYYFDRDDVAL	KNFAKYFLHQS.	HEEREHAE	EKLMKLQNQF	<mark>G</mark> GRIFLQDIKKPE	CDDWESG	:	97
FrogM	:	NYHSDCEAAVNRMLNLELYASYTYSSMYAFFDRDDVAL	HNVAEFFKEHS.	HEEREHAE	EKFMKYQNKF	<mark>G</mark> GRVVLQDIKKPE	CRDEWGNT	:	93
HorseH	:	NYHQDSEAAINRQINLELHASYVYLSMSFYFDRDDVAL	KNFAKYFLHQS.	HEEREHAE	EKLMKLQNQF	<mark>G</mark> GRIFLQDIKKPE	QDDWENG	:	97
RabbitH	:	~~~~~~AINRQINLELYASYVYLSMSYYFDRDDVAL	KNFAKYFLHQS	HEEREHAE	EKLMKLQNQF	<mark>G</mark> GRIFLQDIKKPE	YDDWESG	:	78
MouseH	:	NYHQDAEAAINRQINLELYASYVYLSMSCYFDRDDVAL	KNFAKYFLHQS	H <mark>E</mark> EREHAE	EKLMKLQNQF	<mark>G</mark> GRIFLQDIKKPE	RDDWESG	:	97
Soybean	:	NYADECESAINEQINVEYNASYVYHSLFAYFDRDNVAL	KGFAKFFKESS	EEREHAE	EKLMKYQNTF	<mark>G</mark> GRVVLHPIKNAF	SEFEHVEKGDA	:	173
Maize	:	KFVDDCEAALNEQINVEYNASYAYHSLFAYFDRDNVAL	KGFAKFFKESS	d <mark>e</mark> erehae	EKLMEYQNKF	GRVRLQSIVTPI	TEFDHPEKGDA	:	174
Pea	:	NFADECESVINEQINVEYNASYVYHSLFAYFDRDNVAL	KGFAKFFKESS	EEHREHAE	EKLMKYQNTF	<mark>G</mark> GRVVLHPIKDVF	SEFEHVEKGDA	:	171
Ecoli	:	MLKPEMIEKLNEQMNLELYSSLLYQQMSAWCSYHTF	EGAAAFLRRHA	QEEMTHM	QRLFDYLTDI	GNLPRINTVESPF	AEYSSL	:	83
Cjejuni	:	MLSKEVVKLLNEQINKEMYAANLYLSMSSWCYENSL	DGAGAFLFAHA	SEESDHAR	KKLITYLNEI	DSHVELQEVKQPE	QNFKSL	:	83
Hpylori	:	MLSKDIIKLLNEQVNKEMNSSNLYMSMSSWCYTHSL	DGAGLFLFDHA.	AEEYEHAF	KKLIVFLNEN	NVPVQLTSISAPE	HKFEGL	:	83
Vcholerae	:	MLSQAMVEHLNEQINLEFFSSNLYLQMSAWCEDKGFI	GAAEFLRAHA	VEEMQHMQ	QRLFTYVSEI	GALPILGAIAAPF	RHDFASL	:	83
Ypestis	:	MLKKEMAQKLNEQLNLEFYSANLYLQMSAWCSDKGF	EGAAAFLKKHS	QEEMQHME	ERLFEYLSGI	GSMPVLGTITAPF	VDFASL	:	120
		3-fold	TR	B E	3	4-fold			
SynFtn	:	3-fold Aslmatafomeadttssigsvyalaernsdtrttvfld-	TR -plieAqiqse	B E Dqfayli	3 RVKFANG	4-fold	GQTQRG~~~~	:	182
SynFtn HumanH	:	3-fold Aslmatafqmeadttssiqsvyalaernsdtrttvfld INAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE	TR -plieAqiqse Thylneqvkai	B E DQFAYLIC KELGDHV	3 RVKFANG INLRKMGAPE	<b>4-fold</b> DPTALLVIDNELF SGLAEYLFDKHTI	AGQTQRG~~~~	:	182 183
SynFtn HumanH FrogM	:	3-fold ASLMATAFQMEADTTSSIQSVYALÄERNSDTRTTVFLD LNAMECALHLEKNVNQSLLELHKLÄTDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLÄTDKVDPHLCDFLE	TR -plieAqiqse Thylneqvkai seyleeqvkdi	B E DQFAYLLC KELGDHVI KRIGDFII	<b>3</b> RVKFANG INLRKMGAPE INLKRLGLPE	<b>4-fold</b> DPTALLVIDNELF SGLAEYLFDKHTI NGMGEYLFDKHSV	AGQTQRG~~~~ GDSDNES~~~~ KESS~~~~~	:	182 183 176
SynFtn HumanH FrogM HorseH	:	3-fold ASLMATAFQMEADTTSSIQSVYALAERNSDTRTTVFLI LNAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKVDPHLCDFLE KAMECALHLEKNVNESLLELHKLATDKNDPHLCDFLE	TR -PLIEAQIQSE THYLNEQVKAI SEYLEEQVKDI THYLNEQVKAI	B E DQFAYLLG KELGDHV KRIGDFI KELGDHV	3 RVKFANG NLRKMGAPE NLKRLGLPE NLRRMGAPE	4-fold DPTALLVIDNELR SGLAEYLFDKHTI NGMGEYLFDKHSV SGMAEYLFDKHTI	AGQTQRG~~~~ CDSDNES~~~~ RESS~~~~~ GECDES~~~~	:	182 183 176 182
SynFtn HumanH FrogM HorseH RabbitH	:	3-fold ASLMATAFQMEADTTSSIQSVYALAERNSDTRTVFLD INAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKNDPHLCDFIE IKAMECALHLEKNVNESLLELHKLATDKNDPHLCDFIE INAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE	TR -PLIEAQIQSE THYLNEQVKAI SEYLEEQVKDI THYLNEQVKAI THYLNEQVKSI	B E DQFAYLIG KELGDHVI KRIGDFII KELGDHVI KELGDHVI	3 RVKFANG NLRKMGAPE NLRRMGAPE NLRRMGAPE	4-fold DPTALLVIDNELF SSCLAEYLFDKHTI NGMGEYLFDKHSV SSGMAEYLFDKHTI	GQTQRG~~~~ GDSDNES~~~~ KESS~~~~~ GECDES~~~~ GHSDNES~~~~	:	182 183 176 182 164
SynFtn HumanH FrogM HorseH RabbitH MouseH	: : : : : : : : : : : : : : : : : : : :	3-fold ASLMATAFQMEADTTSSIQSVYALAERNSDTRTTVFLD INAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKNDPHLCDFIE LNAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE INAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE	TR -PLIEAQIQSE THYLNEQVKAI SEYLEEQVKDI THYLNEQVKAI THYLNEQVKSI	B E DQFAYLIG KELGDHV KRIGDFI KELGDHV KELGDHV KELGDHV	3 RVKFANG NLRKMGAPE NLKRLGLPE NLRRMGAPE NLRKMGAPE	4-fold DPTALLVIDNELF SSGLAEYLFDKHTI NGMGEYLFDKHTI SSGMAEYLFDKHTI AGMAEYLFDKHTI	GOTORG~~~~ GDSDNES~~~~ KESS~~~~ GECDES~~~~ GHSDNES~~~~ GHGDES~~~~	:	182 183 176 182 164 182
SynFtn HumanH FrogM HorseH RabbitH MouseH Soybean	:::::::::::::::::::::::::::::::::::::::	3-fold ASLMATAFQMEADTTSSIQSVYALAERNSDTRTTVFLD INAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKNDPHLCDFIE LNAMECALHLEKNVNESLLELHKLATDKNDPHLCDFIE LNAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LYAMELALSLEKLVNEKLLNVHSVADRNNDPQMADFIE	TR -PLIEAQIQSE THYLNEQVKAT SEYLEEQVKAT THYLNEQVKAT THYLNEQVKST TYYLSEQVKST SEFLSEQVEST	B E DQFAYLIG KELGDHV KRIGDFI KELGDHV KELGDHV KELGDHV	3 RVKFANG NLRKMGAPE NLRRMGAPE NLRKMGAPE NLRKMGAPE QLRRVGK	4-fold DPTALLVIDNELF SGLAEYLFDKHTI NGMGEYLFDKHSV SGMAEYLFDKHTI SGMAEYLFDKHTI - GHGVWHFDQRLI	AGQTQRG~~~~ GDSDNES~~~~ GECDES~~~~ GHSDNES~~~~ GHGDES~~~~ D~~~~	:	182 183 176 182 164 182 250
SynFtn HumanH FrogM HorseH RabbitH MouseH Soybean Maize	:::::::::::::::::::::::::::::::::::::::	3-fold ASLMATAFQMEADTTSSIQSVYALÄERNSDTRTTVFLD LNAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKNDPHLCDFIE LKAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LNAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LYAMELALSLEKLVNEKLLNVHSVARNNDPQMADFIE LYAMELALALEKLVNEKLHNHGVATRCNDPQLTDFIE	TR -PLIEAQIQSE THYLNEQVKAI SEYLEEQVKDI THYLNEQVKAI THYLNEQVKSI SEFLSEQVESI SEFLEEQGEAI	B E DQFAYLI( KELGDHV KRIGDFI KELGDHV KELGDHV KKISEYV/ NKISKYV/	3 RVKFANG INLRKMGAPE INLRKRLGLPE INLRKMGAPE INLRKMGAPE QLRRVGK QLRRVGK	4-fold DPTALLVIDNELF SGLAEYLFDKHTI NGMGEYLFDKHTI SGMAEYLFDKHTI AGMAEYLFDKHTI -GHGVWHFDQMLI -GHGVWHFDQMLI	AGQTQRG~~~~ GDSDNES~~~~ GECDES~~~~ GHSDNES~~~~ GHGDES~~~~ D~~~~~ EEEA~~~~~	:::::::::::::::::::::::::::::::::::::::	182 183 176 182 164 182 250 254
SynFtn HumanH FrogM HorseH RabbitH MouseH Soybean Maize Pea	:::::::::::::::::::::::::::::::::::::::	3-fold ASLMATAFQMEADTTSSIQSVYALAERNSDTRTTVFLI LNAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKNDPHLCDFIE LKAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LNAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LYAMELALSLEKLVNEKLLNVHSVADRNNDPQMADFIE LYAMELALSLEKLVNEKLHNLHGVATRCNDPQLTDFIE LYAMELALSLEKLTNEKLLNVHSVAERNNDLEMTHFIE	TR -PLIEAQIQSE THYLNEQVKAI SEYLEEQVKDI THYLNEQVKAI THYLNEQVKSI TYYLSEQVKSI SEFLEEQGEAI GEYLAEQVEAI	B E DQFAYLIQ KELGDHV KELGDHV KELGDHV KELGDHV KKISEYVZ NKISKYVZ KKISEYVZ	3 NLRKMGAPE NLRKMGAPE NLRRMGAPE NLRKMGAPE QLRRVGK QLRRVGK	4-fold DPTALLVIDNELF SGLAEYLFDKHTI SGMAEYLFDKHTI SGMAEYLFDKHTI SGMAEYLFDKHTI -GHGVWHFDQRLI -GHGVWHFDQRLI -GHGVWHFDQRLI	AGQTQRG~~~~ GDSDNES~~~~ GECDES~~~~ GHSDNES~~~~ GHGDES~~~~ BEEA~~~~~ BEEA~~~~~ HGVHGA~~~~	:::::::::::::::::::::::::::::::::::::::	182 183 176 182 164 182 250 254 253
SynFtn HumanH FrogM HorseH RabbitH MouseH Soybean Maize Pea Ecoli	:::::::::::::::::::::::::::::::::::::::	3-fold ASLMATAFQMEADTTSSIQSVYALAERNSDTRTTVFLD LNAMECALHLERNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKNDPHLCDFIE LNAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LNAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LYAMELALSLEKLVNEKLLNVHSVAPRNNDPQMADFIE LYAMELALSLEKLTNEKLLNVHSVAPRNNDLEMTHFIE DELFCBTYKHEQLITQKINELAHAAMTNQDYPTFNFLO	TR -PLIEAQIQSE THYLNEQVKAI SEYLEEQVKDI THYLNEQVKSI THYLSEQVKSI SEFLEQUESI SEFLEEQGEAI GEYLAEQVEAI -WYVSEQHEEE	B E DQFAYLIG KELGDHV KRIGDFI KELGDHV KELGDHV KKISEYV KKISEYV KKISEYV KLFKSTI	RVKFANG INLRKMGAPE INLRKMGAPE INLRKMGAPE INLRKMGAPE QLRRVGK QLRRVGK KLSLAGK	4-fold DPTALLVIDNELF SGLAEYLFDKHTI NGMGEYLFDKHTI SGMAEYLFDKHTI AGMAEYLFDKHTI -GHGVWHFDQMLI -GHGVWHFDQMLI -GGVWHFDQMLI SGEGLYFIDKELS	AGQTQRG~~~~ GDSDNES~~~~ GECDES~~~~~ GHSDNES~~~~ GHGDES~~~~~ BCEA~~~~~ EEEA~~~~~ TLDTQN~~~~~		182 183 176 182 164 182 250 254 253 165
SynFtn HumanH FrogM HorseH RabbitH MouseH Soybean Maize Pea Ecoli Cjejuni		3-fold ASLMATAFQMEADTTSSIQSVYALAERNSDTRTTVFLD LNAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKNDPHLCDFIE LKAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LNAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LYAMELALSLEKLVNEKLLNVHSVADRNNDPQMADFIE LYAMELALSLEKLVNEKLLNVHSVADRNNDPQLTDFIE LYAMELALSLEKLVNEKLHNLHGVATRCNDPQLTDFIE DELFQETYKHEQLITQKINELAHAAMTNQDYPTFNFLQ LDVFEKTYEHEQFITKSINTLVEHMLTHKDYSTFNFLQ	TR -PLIEAQIQSE THYINEQVKAI SEYLEEQVKDI THYINEQVKAI THYLNEQVKSI SEFLSEQVESI SEFLSEQUESI SEFLEEQGEAI GEYLAEQVEAI -WYVSEQHEEE -WYVSEQHEEE	B E DQFAYLIG KELGDHV KELGDHV KELGDHV KKISEYVZ KKISEYVZ KKISEYVZ KKISEYVZ	RVKFANG NIRKMGAPE NIRKMGAPE NIRKMGAPE NIRKMGAPE QIRRVGK QIRRVGK QIRRVGK QIRRVGK XISLIAGK KISLIAGK	4-fold DPTALLVIDNELF SGLAEYLFDKHTI NGMGEYLFDKHTI SGMAEYLFDKHTI SGMAEYLFDKHTI -GHGVWHFDQRLI -GHGVWHFDQRLI SGEGLYFIDKELS HGNGLYLADQYIK	AGQTQRG~~~~ GDSDNES~~~~ GECDES~~~~ GHSDNES~~~~ D~~~~~ BEEA~~~~~ HGVHGA~~~~~ NIALSKKK~~~		182 183 176 182 164 182 250 254 253 165 167
SynFtn HumanH FrogM HorseH RabbitH MouseH Soybean Maize Pea Ecoli Cjejuni Hpylori		3-fold ASLMATAFQMEADTTSSIQSVYALÄERNSDTRTTVFLD LNAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKNDPHLCDFIE LKAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LNAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LYAMELALSLEKLVNEKLLNVHSVARNNDPQMADFIE LYAMELALSLEKLVNEKLLNVHSVARNNDLEMTHFIE DELFQETYKHEQLITQKINELAHAAMNNQDYPTNFLQ LDVFEKTYEHEQFITKSINTLVEHMLTHKDYSTFNFLQ TQIFQKAYEHEGHISESINNIVDHAIKGKDHATFNFLQ	TR -PLIEAQIQSE THYINEQVKAI SEYLEEQVKDI THYLNEQVKAI THYLNEQVKSI SEFLSEQVESI SEFLEEQGEAI GEYLAEQVEAI -WYVSEQHEEE -WYVSEQHEEE -WYVSEQHEEE	B E DQFAYLIQ KELGDHV KELGDHV KELGDHV KELGDHV KKISEYV KKISEYV KKISEYV LFKSII LFRGIV	RVKFANG RURRMGAPE NLRRMGAPE NLRRMGAPE NLRKMGAPE QLRRVGK QLRRVGK KLSLAGK KIKLIGE KIELIGN	4-fold DPTALLVIDNELF SGLAEYLFDKHTI NGMGEYLFDKHTI SGMAEYLFDKHTI -GHGVWHFDQRLI -GHGVWHFDQRLI SGEGLYFIDKELS HGNGLYLADQYIK ENHGLYLADQYIK	AGQTQRG~~~~ GDSDNES~~~~ KESS~~~~~ GECDES~~~~ GHSDNES~~~~ D~~~~~ EEEA~~~~~ TLDTQN~~~~ GIAKSRKS~~~ GIAKSRKS~~~		182 183 176 182 164 182 250 254 253 165 167 167
SynFtn HumanH FrogM HorseH RabbitH MouseH Soybean Maize Pea Ecoli Cjejuni Hpylori Vcholerae		3-fold ASLMATAFQMEADTTSSIQSVYALAERNSDTRTTVFLD INAMECALHLEKNVNQSLLELHKLATDKNDPHLCDFIE LEAMQAALQLEKTVNQALLDLHKLATDKNDPHLCDFIE LKAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE INAMECALHLEKSVNQSLLELHKLATDKNDPHLCDFIE LYAMELALSLEKLVNEKLLNVHSVADRNNDPQMADFIE LYAMELALSLEKLVNEKLLNVHSVADRNNDPQMADFIE DELFQETYKHEQLITQKINELAHAAMTNQDYPTNFLO DUFEKTYEHEQFITKSINTLVEHMLTHKDYSTNFLO TQIFQKAYEHEQHISESINNIVDHAIKGKDHATFNFLO	TR -PLIENQIQSE THYLNEQVKAT SEYLEPQVKJI THYLNEQVKSI TYYLSEQVKSI SEFLEPQGEAI GEYLAPQVEAI -WYVSEQHEEE -WYVSEQHEEE -WYVSEQHEEE -WYVSEQHEEE -WYVSEQHEEE	B E DQFAYLIX KELGDHV KELGDHV KELGDHV KELGDHV KKISEYV KKISEYV KKISEYV KLFKSIII ALFRGIVI KLFKGIII	RVKFANG NLRKMGAPE NLRRMGAPE NLRRMGAPE NLRKMGAPE QLRRVGK QLRRVGK KLSLAGK KISLAGK KISLAGK KIELIGN KLELVGE	4-fold DPTALLVIDNELF SGLAEYLFDKHTI SGMAEYLFDKHTI SGMAEYLFDKHTI SGMAEYLFDKHTI -GHGVWHFDQRLI -GHGVWHFDQRLI SGEGLYFIDKELS HGNGLYLADQYIK ENHGLYLADQYIK DGKALFFIDKDLA	AGQTQRG~~~~ GDSDNES~~~~ GECDES~~~~ GHSDNES~~~~ GHGDES~~~~ EEEA~~~~~ TLDTQN~~~~~ NIALSRKK~~~ ALAKKGSSSVM		182 183 176 182 164 182 250 254 253 165 167 167 170

**Figure S1.** Comparison of the sequence of *Syn*Ftn with those of selected other ferritins. The area shaded in blue denotes those residues that comprise the 3-fold channel with the positions of the two conserved carboxylates of the animal proteins marked by blue triangles. The area shaded orange denotes the residues that comprise the 4-fold channel. Red shading indicates residues that line the B-channel of prokaryotic ferritins and green shading the positions of the 'transfer carboxylates' identified in Frog M and Human H ferritin. The proteins shown are ferritin heavy chain from Homo sapiens (HumanH), ferritin middle subunit from *Rana catesbeiana* (FrogM), ferritin heavy chain from *Equus caballus* (HorseH), ferritin heavy chain from *Oryctolagus cuniculus* (RabbitH), ferritin 1 from *Zea mays* (Maize), ferritin 1 from *Pisum sativum* (Pea), non-heme ferritin FtnA from *Escherichia coli* (Ecoli), non-heme ferritin from *Vibrio cholera* (Vcholerae) and ferritin from *Yersinia pestis* (Ypestis).



**Figure S2.** The B-channel of *Syn*Ftn is capped by an N terminal extension of the peptide chain. The image shows the B-channel of *Syn*Ftn formed at the intersection of three subunit monomers (PDB entry 6GKA) overlaid with an orange sphere at the position at which an iron ion was observed in the structure of E44Q *Pm*Ftn soaked overnight in a Fe<sup>2+</sup> containing solution (PDB entry 4ZKH). The brown mesh represents the surface of the N terminal of *Syn*Ftn, which is an extension relative to *E. coli* FtnA, the most extensively characterized of the prokaryotic Ftn proteins.



**Figure S3.** Comparison of the crystal structure of wild type and variant D137A *Syn*Ftn. Oxygen atoms coloured red, nitrogen blue, sulphur orange and carbon cyan (wild type) or white (D137A). The position of the mutated residue is marked by the red elipse.



**Figure S4**. The ferroxidase center of Fe<sup>2+</sup>-soaked *Syn*Ftn variants. The di-iron site of D137A *Syn*Ftn following the soaking of crystals in a 5 mM Fe<sup>2+</sup> solution for either 2 min (A) or 20 min (B). (C-D) as (A-B) but for crystals of E62A *Syn*Ftn. Carbon is shown in white, nitrogen in blue, oxygen in red and iron as bright orange spheres with associated water molecules or (hydr)oxide ions as red spheres. The light green mesh in each of panels A to D shows the anomalous difference Fourier map calculated from data collected at the iron K-edge and contoured at 8  $\sigma$ .



**Figure S5.** Inhibition of *Syn*Ftn by Zn<sup>2+</sup>. The increase in absorbance at 340 nm as a function of time following the addition of 400 equivalents of Fe<sup>2+</sup> to 0.5  $\mu$ M protein (24mer). The response of wild type protein is shown in black and variant D137A in red, solid lines represent data for addition of Fe<sup>2+</sup> to apo proteins and open circles for addition of Fe<sup>2+</sup> to protein pre-incubated with 96 equivalents of Zn<sup>2+</sup> (4 Zn<sup>2+</sup>/monomer). All measurements were carried out in MES pH 6.5 at 25 °C.



**Figure S6.** EPR properties of *Syn*Ftn and variants . Full sweep EPR spectra of: wild type *Syn*Ftn prior to the addition of  $Fe^{2+}$  (black); wild type *Syn*Ftn frozen 9 s after the addition of 72 equivalents of  $Fe^{2+}$  (red); variant E62A *Syn*Ftn frozen 12 s after the addition of 72 equivalents of  $Fe^{2+}$  (blue); and, variant D137A *Syn*Ftn frozen 20 s after the addition of 72 equivalents of  $Fe^{2+}$  (orange). Note that the intensity of the radical signal centred on 3386 G is variable between preparations and does not correlate with the E62A and D137A substitutions.



**Figure S7.** Comparison of the crystal structure of wild type and variant E62A *Syn*Ftn. Oxygen atoms coloured red, nitrogen blue, sulphur orange and carbon cyan (wild type) or white (E62A). The position of the mutated residue is marked by the red elipse.

PDB code	6SOM	6SON	6500	
Fe <sup>2+</sup> Soak (min)	0	2	20	
Wavelength	0.9763	0.9795	0.9795	
Resolution range	40.55 - 2.15 (2.23 - 2.15)	51.07 - 1.60 (1.66 - 1.60)	44.12 - 1.57 (1.63 - 1.57)	
Space group	F 4 3 2	F 4 3 2	F 4 3 2	
Unit cell	176.8 176.8 176.8 90 90 90	176.9 176.9 176.9 90 90 90	176.5 176.5 176.5 90 90 90	
Total reflections	223008 (22708)	609753 (61303)	852797 (83644)	
Unique reflections	13408 (1302)	31808 (3112)	33428 (3301)	
Multiplicity	16.6 (17.4)	19.2 (19.7)	25.5 (25.3)	
Completeness (%)	99.81 (100.00)	99.93 (100.00)	99.96 (99.97)	
Mean I/sigma(I)	10.25 (1.47)	26.10 (1.58)	27.06 (1.27)	
Wilson B-factor	37.51	30.84	29.22	
R-merge	0.1991 (1.465)	0.05239 (1.774)	0.06179 (2.264)	
R-meas	0.2055 (1.509)	0.05386 (1.821)	0.06308 (2.31)	
R-pim	0.05005 (0.3592)	0.01231 (0.4085)	0.01252 (0.4565)	
CC1/2	0.997 (0.693)	1 (0.644)	1 (0.605)	
CC*	0.999 (0.905)	1 (0.885)	1 (0.868)	
Reflections used in refinement	13406 (1302)	31807 (3112)	33427 (3300)	
Reflections used for R-free	677 (65)	1539 (153)	1671 (165)	
R-work	0.1855 (0.2561)	0.1582 (0.2303)	0.1652 (0.2448)	
R-free	0.2397 (0.3310)	0.1890 (0.2805)	0.1957 (0.2966)	

 Table S1. SynFtn D137A mutant. Data collection and refinement statistics.

CC(work)	0.957 (0.859)	0.968 (0.868)	0.959 (0.853)	
CC(free)	0.946 (0.752)	0.948 (0.810)	0.956 (0.768)	
Number of non- hydrogen atoms	1514	1630	1583	
Macromolecules	1393	1416	1399	
Ligands	1	3	2	
Solvent	120	211	182	
Protein residues	178	178	178	
RMS(bonds)	0.006	0.005	0.005	
RMS(angles)	0.71	0.69	0.67	
Ramachandran favored (%)	98.86	100.00	100.00	
Ramachandran allowed (%)	1.14	0.00	0.00	
Ramachandran outliers (%)	0.00	0.00	0.00	
Rotamer outliers (%)	0.00	0.66	0.00	
Clashscore	1.83	1.79	0.73	
Average B-factor	37.46	34.81	32.62	
Macromolecules	36.95	33.02	31.18	
Ligands	43.73	51.64	43.96	
Solvent	43.35	46.58	43.56	

Statistics for the highest-resolution shell are shown in parentheses.

PDB code	6SOP	6SOQ	6SOR	
Fe <sup>2+</sup> Soak (min)	0	2	20	
Wavelength	0.9795	0.9795	0.9795	
Resolution range	40.58 - 1.93 (2.00 - 1.93)	36.13 - 1.67 (1.73 - 1.67)	39.46 - 1.74 (1.80 - 1.74)	
Space group	F 4 3 2	F 4 3 2	F432	
Unit cell	176.9 176.9 176.9 90 90 90	177 177 177 90 90 90	176.5 176.5 176.5 90 90 90	
Total reflections	128714 (12505)	211108 (13333)	194099 (16168)	
Unique reflections	18381 (1789)	27809 (2527)	24709 (2426)	
Multiplicity	7.0 (7.0)	7.6 (5.3)	7.9 (6.7)	
Completeness (%)	99.82 (99.89)	98.90 (91.85)	99.87 (100.00)	
Mean I/sigma(I)	11.11 (1.19)	22.75 (1.44)	15.09 (1.52)	
Wilson B-factor	35.55	28.17	30.79	
R-merge	0.1016 (1.327)	0.04735 (0.9856)	0.06909 (0.9414)	
R-meas	0.1098 (1.435)	0.05083 (1.093)	0.07412 (1.021)	
R-pim	0.04087 (0.5372)	0.01811 (0.4579)	0.02631 (0.3896)	
CC1/2	0.998 (0.49)	0.999 (0.496)	0.998 (0.581)	
CC*	0.999 (0.811)	1 (0.814)	1 (0.857)	
Reflections used in refinement	18379 (1789)	27808 (2526)	24708 (2426)	
Reflections used for R-free	931 (90)	1398 (120)	1248 (119)	
R-work	0.1550 (0.2735)	0.1511 (0.2555)	0.1575 (0.2304)	
R-free	0.1998 (0.3269)	0.1830 (0.3370)	0.1873 (0.2812)	

 Table S2.
 SynFtn E62A mutant. Data collection and refinement statistics.

CC(work)	0.967 (0.802)	0.969 (0.810)	0.968 (0.844)	
CC(free)	0.964 (0.755)	0.945 (0.645)	0.966 (0.836)	
Number of non- hydrogen atoms	1583	1637	1600	
Macromolecules	1395	1398	1395	
Ligands	1	4	4	
Solvent	187	235	201	
Protein residues	178	178	178	
RMS(bonds)	0.007	0.007	0.007	
RMS(angles)	1.01	1.09	1.02	
Ramachandran favored (%)	99.43	99.43	100.00	
Ramachandran allowed (%)	0.57	0.57	0.00	
Ramachandran outliers (%)	0.00	0.00	0.00	
Rotamer outliers (%)	0.00	0.00	0.00	
Clashscore	1.46	2.18	1.46	
Average B-factor	37.11	30.13	33.13	
Macromolecules	35.79	28.09	31.68	
Ligands	39.32	30.23	32.88	
Solvent	46.95	42.27	43.21	

Statistics for the highest-resolution shell are shown in parentheses.

**Table S3.** Refined fractional occupancies of metal binding sites in *Syn*Ftn mutants. Soak solutions comprised the well solution with 5 mM Fe<sup>2+</sup> and the pH adjusted to 6.5. Crystals were soaked for either 2 or 20 min prior to freezing.

SvnEtn variant	Soak/min	Fracti	onal occupa	occupancy <sup>a</sup>	
		Site A	Site B	Site 3FC	
D137A	2	0.50	0.32	0	
	20	0.56	0.54	0	
E62A	2	0.74	0.51	0.26	
	20	0.92	0.88	0.33	

<sup>a</sup> Sites A and B constitute the ferroxidase centre. Site 3FC represents the iron binding site which lies on the symmetry axis in the 3-fold channel of the iron-soaked wild type ferritin structure (PDB entry 3OUY), and has a maximal occupancy of 0.33