Supporting Information for:

Native Electron Capture Dissociation Maps to Iron-Binding Channels in Horse Spleen Ferritin

Owen S. Skinner[†]^{‡*}, Michael O. McAnally[†][‡], Richard P. Van Duyne[†], George C. Schatz[†], Kathrin Breuker[§], Philip D. Compton[†] and Neil L. Kelleher^{†*}

† Department of Chemistry, Northwestern University, Evanston IL, USA§ Institute of Organic Chemistry, University of Innsbruck, Innsbruck, AUSTRIA.

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Table S-1. Masses and errors for the matched fragment ions displayed in fragment maps: "Supp_Table_1.xlsx".

S S Q I R Q N Y S T E V E A A V N R L V N L V N L Y L R 25 26 A S Y T Y L S L G F Y F D R D D V A L E G V H F 50 51 F R E L A E E K R E G A E R L L K M Q N Q R G G R 75 76 A L F Q D L Q K P S Q D E W G T T L D A M K A A I 100 101 V L E K S L N Q A L L D L H A L G S A Q A D P H L 125 126 C D F L E S H F L D E E V K L I K M G D H L T N 150 151 I Q R L V G S Q A G L G E Y L F E R L T L K H D C

Figure S-1. Graphical fragment map representation of fragment ions formed from quadrupolebased isolation and CAD dissociation of the 14+ L-chain ejected from the intact ferritin complex. Only *b*- and *y*-type fragment ions were detected. The red square corresponds to an N-terminal acetylation modification and the orange square corresponds to a cysteine methyl disulfide modification.

Ν	S	S	Q	I	R	Q	Ν	Y	S	т	E	v	E	A	A	V	Ν	R	L	v	Ν	L	Y	L	R	25
26	A	S	Y	т	Y	L	S	L	G	F	Y	F	D	R	D	D	V	Α	L	Ε	G	V	C	Н	F	50
51	F	R	Ε	L	A	Ε	E	K	R	E	G	A	Ε	R	L	L	Κ	Μ	Q	Ν	Q	R	G	G	R	75
76	A	L	F	Q	D	L	Q	К	Ρ	S	Q	D	E	w	G	т	т	L	D	A	М	K	A	A	I	100
101	V	L	E	K	S	L	Ν	Q	A	L	L	D	L	н	A	L	G	S	Α	Q	A	D	Ρ	Н	L	125
126	C	D	F	L	Ε	S	Н	F	L	D	E	E	V	Κ	L	I	K	K	Μ	G	D	Η	L	т	Ν	150
151	Ι	Q	R	L	V	G	S	Q	Α	G	L	G	Ε	Y	L	F	Е	R	L	т	L	Κ	н	D	С	

Figure S-2. Evidence of NECD *c*-fragment ions from the L-chain obtained from quadrupolebased isolation and activation of the intact ferritin complex. These product ions must therefore be formed after quadrupole isolation.

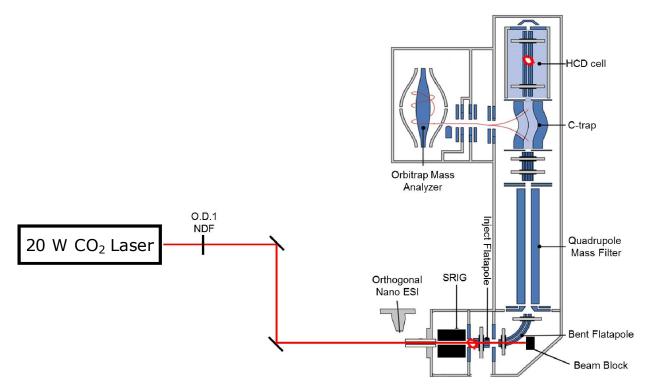


Figure S-3. Experimental setup for front-end infrared excitation (FIRE). The laser is aligned collinearly through the inlet capillary of the instrument, and blocked by a beam block placed behind the bent flatapole. The MS-portion of this figure was modified from Ref. 6.

N	G	D	V	E	K	G	K	K	I	F	V	Q	K	C	A	Q	С	Η	Т	V	E	K	G	G	K	25
26	н	К	Т	G	Ρ	Ν	L	Н	G	L	F	G	R	K	Т	G	Q	A	Ρ	G	F	Т	Y	Т	D	50
51	A	ĮΝ	K	N	K	G	I	Т	w	К	E	Ε	т	L	Μ	Ε	Y	L	E	Ν	Ρ	K	К	Y	I	75
76	Ρ	G	Т	К	М	I	F	A	G	I	K	К	К	т	Ε	R	Ε	D	L	I	A	Y	L	К	K	100
101	A	т	Ν	Ε	С																					

Figure S-4. Fragment map showing the NECD *c*- and complementary *y*-type cleavage products of cytochrome *c* upon laser activation by FIRE. The orange box corresponds to the mass shift caused by the covalent heme binding (+615.1695 Da), which is also bound to Cys-17. No fragment ions were observed without laser activation (data not shown).

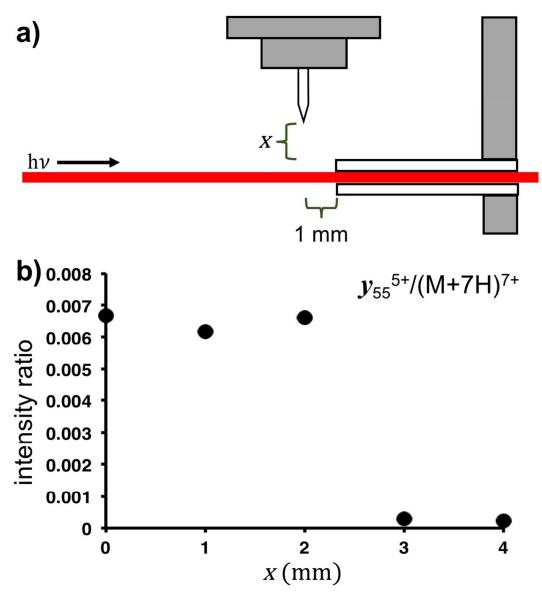


Figure S-5. (a) Experimental schematic of the FIRE setup highlighting the distance parameters used; *x* is the variable tip-laser distance. (b) The relative ratio of the y_{55} 5+ compared to the 7+ molecular ion of cytochrome *c* at varying electrospray emitter distances. The y_{55} fragment is an abundant NECD product ion and its intensity after normalization to the molecular ion is used to indicate the extent of NECD cleavage.

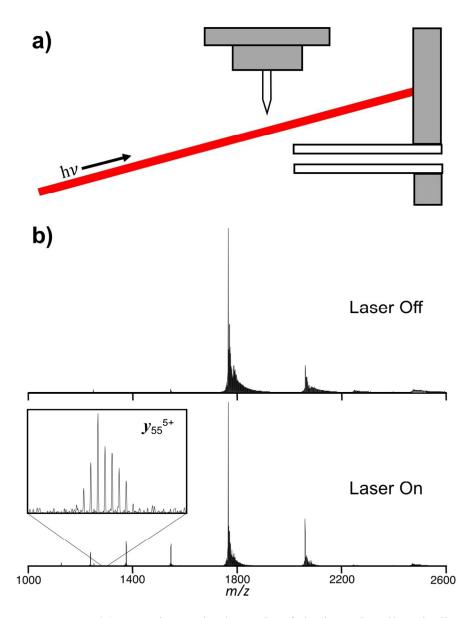


Figure S-6. (a) Experimental schematic of the intentionally misaligned FIRE setup. (b) Spectra of cytochrome c with the laser off (top) and on (bottom). Laser irradiation with FIRE produced evidence of higher-charged monomers and NECD fragmentation, despite only overlapping with the sample just after exit from the electrospray tip. Thus, the majority of ion activation from FIRE occurs in the droplet.

 S
 S
 Q
 I
 R
 Q
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 Y
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 V
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 A
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 L
 V
 L
 R
 25

 26
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Figure S-7. Fragment map of ferritin activated by FIRE exhibiting *c*-fragment ions.

N	S	S	Q	I	R	Q	Ν	Y	S	т	Е	V	Ε	Α	Α	V	Ν	R	L	V	Ν	L	Y	L	R	25
26	A	S	Y	т	Y	L	S	L	G	F	Y	F	D	R	D	D	V	Α	L	E	G	V	C	Н	F	50
51	F	R	Ε	L	A	Ε	Ε	K	R	Ε	G	A	Ε	R	L	L	Κ	Μ	Q	Ν	Q	R	G	G	R	75
76	A	L	F	Q	D	L	Q	K	Ρ	S	Q	D	Ε	W	G	т	т	L	D	A	Μ	К	Α	A	I	100
101	V	L	E	K	S	L	Ν	Q	A	L	L	D	L	Н	A	L	G	S	Α	Q	A	D	Ρ	Н	L	125
126	С	D	F	L	E	S	н	F	L	D	Ε	Ε	V	К	L	I	К	K	Μ	G	D	Н	L	Т	Ν	150
151	I	Q	R	L	V	G	S	Q	A	G	L	G	Ε	Y	L	F	Ε	R	L	Т	L	K	н	D	С	

Figure S-8. Activation of the apo-form of horse spleen ferritin produced similar *c*-fragments to the holo-form, suggesting that the iron mineral core is not implicated in NECD.