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".

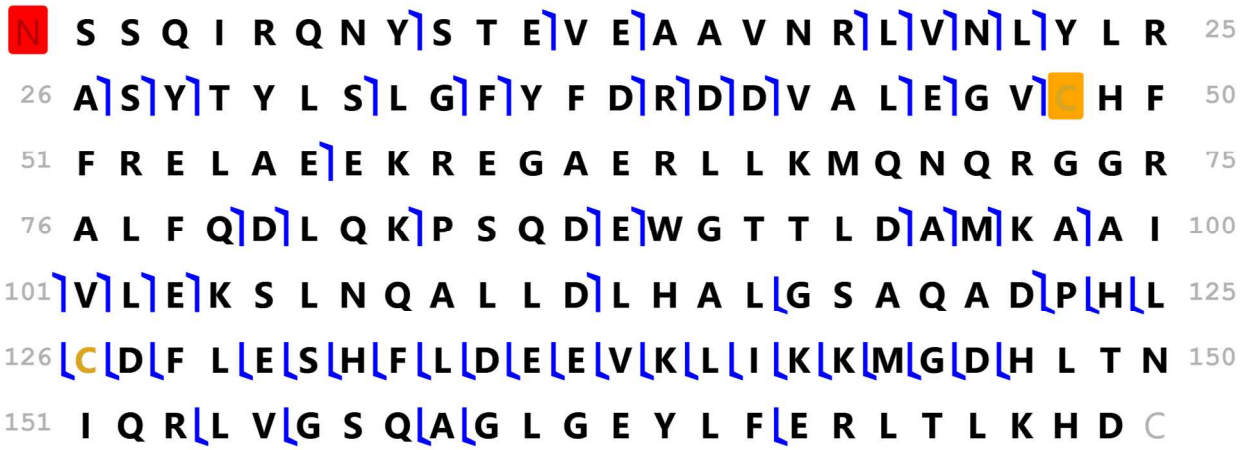

Figure S-1. Graphical fragment map representation of fragment ions formed from quadrupole-based isolation and CAD dissociation of the 14+ L-chain ejected from the intact ferritin complex. Only *b*- and *y*-type fragment ions were detected. The red square corresponds to an N-terminal acetylation modification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

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

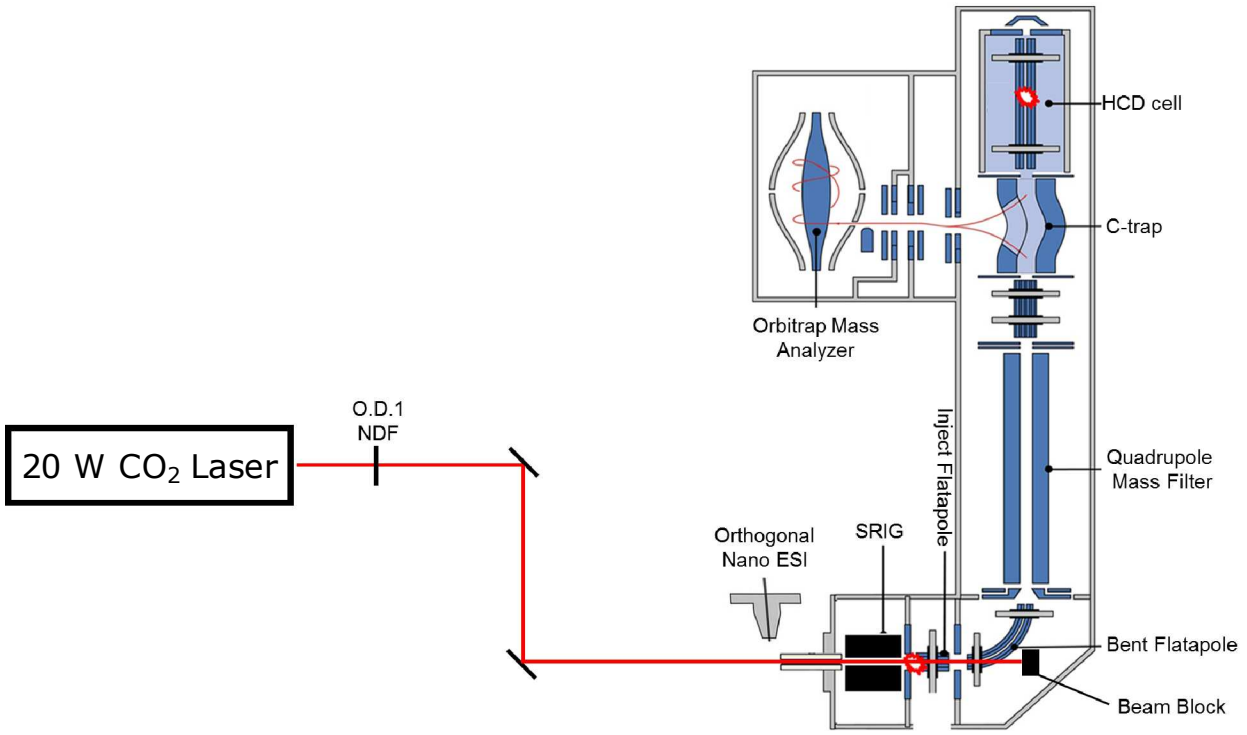

Figure S-3. Experimental setup for front-end infrared excitation (FIRE). The laser is aligned collinearly through the inlet capillary of the instrument, and blocked by a beam block placed behind the bent flatapole. The MS-portion of this figure was modified from Ref. 6.

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).

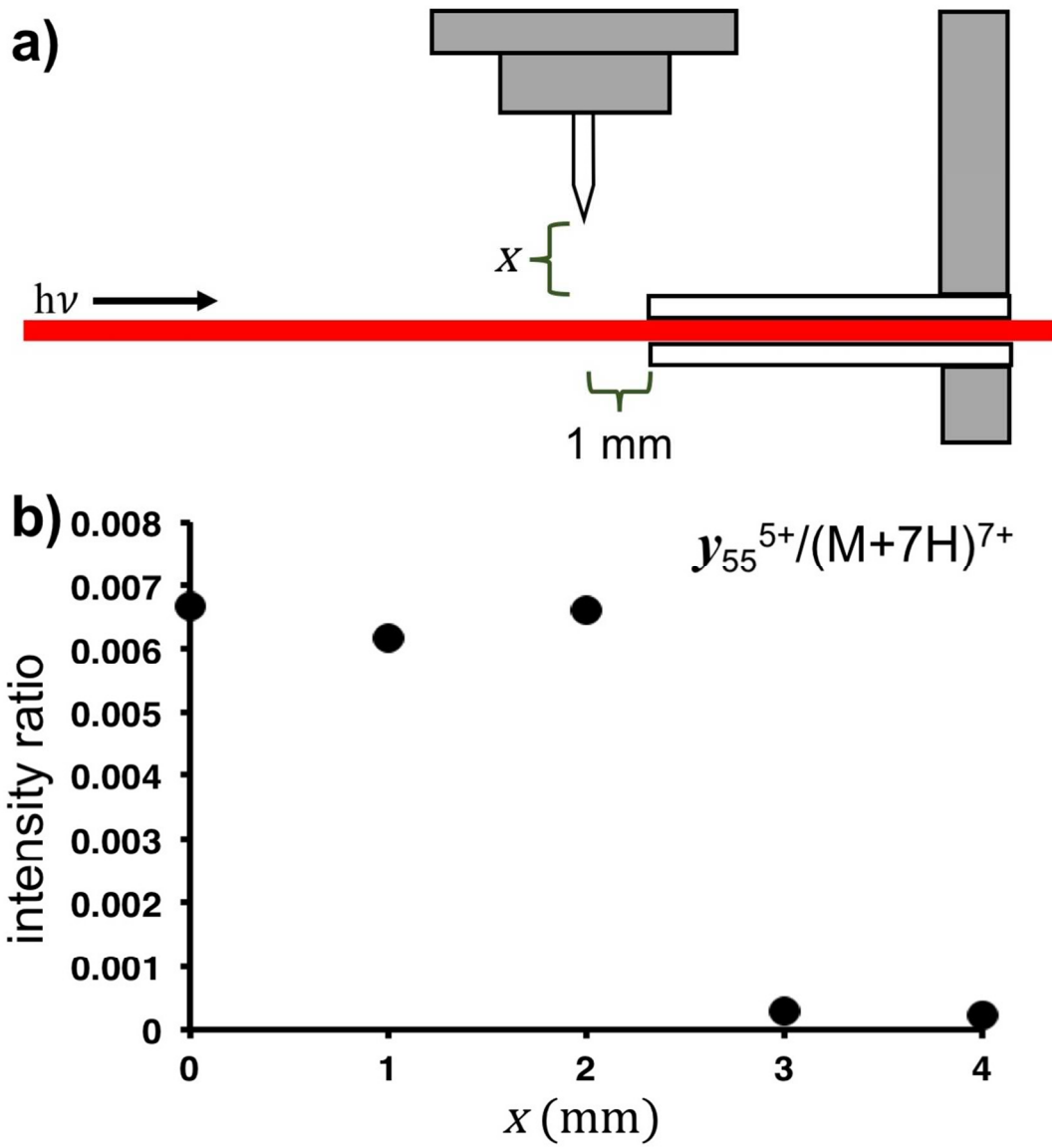

Figure S-5. (**a**) Experimental schematic of the FIRE setup highlighting the distance parameters used; x is the variable tip-laser distance. (**b**) The relative ratio of the $\boldsymbol{y}_{55}$ 5+ compared to the 7+ molecular ion of cytochrome *c* at varying electrospray emitter distances. The $\boldsymbol{y}_{55}$ 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.

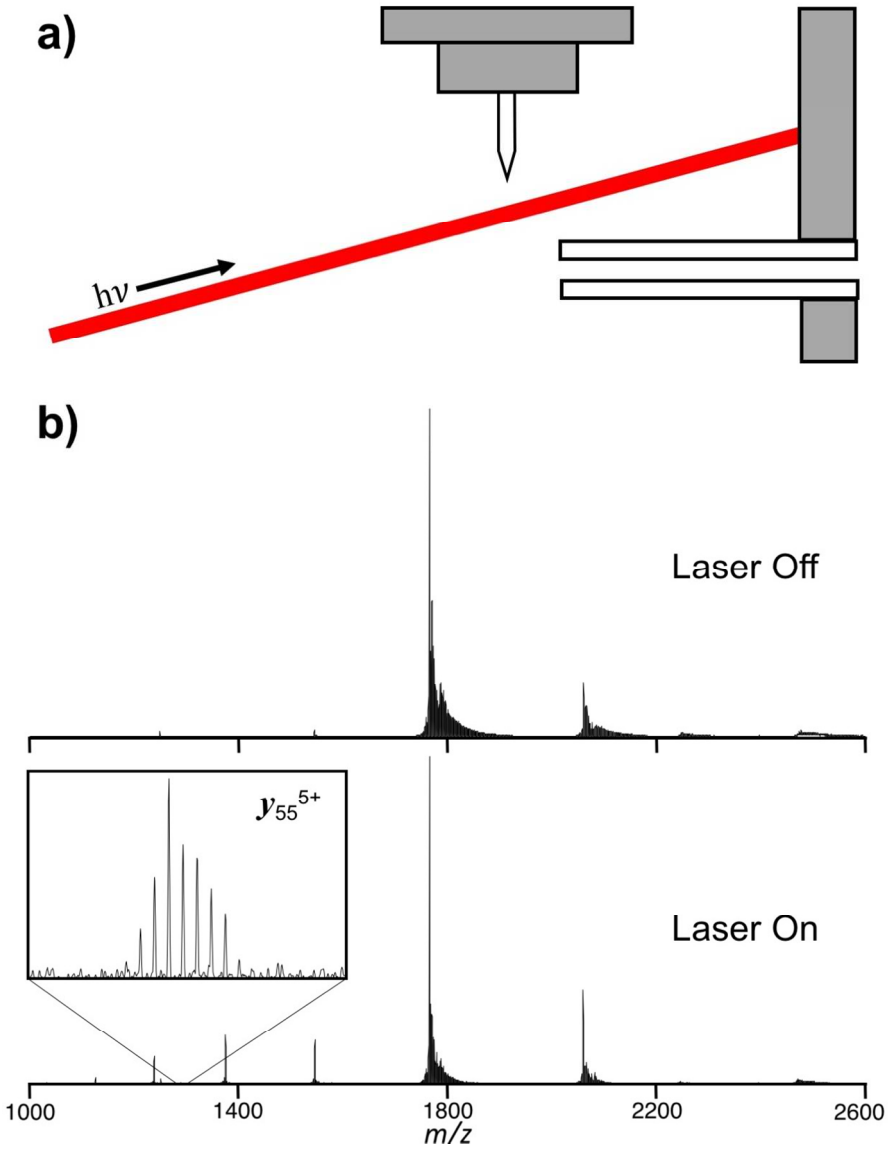

Figure S-6. (**a**) Experimental schematic of the intentionally misaligned FIRE setup. (**b**) Spectra of cytochrome *c* with the laser off (top) and on (bottom). Laser irradiation with FIRE produced evidence of higher-charged monomers and NECD fragmentation, despite only overlapping with the sample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

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

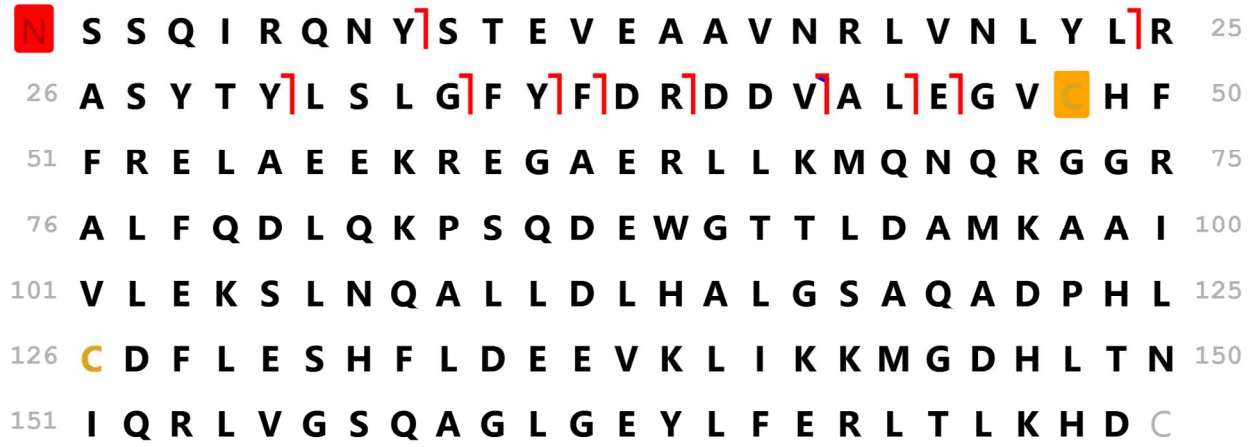

Figure S-8. Activation of the apo-form of horse spleen ferritin produced similar *c*-fragments to the holo-form, suggesting that the iron mineral core is not implicated in NECD.