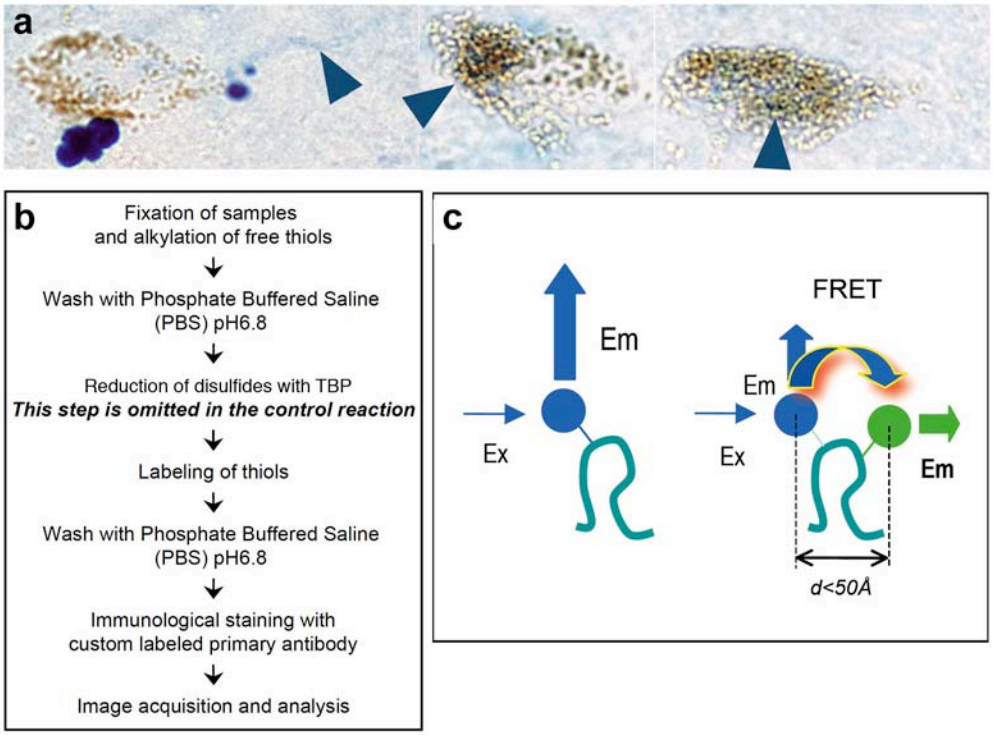
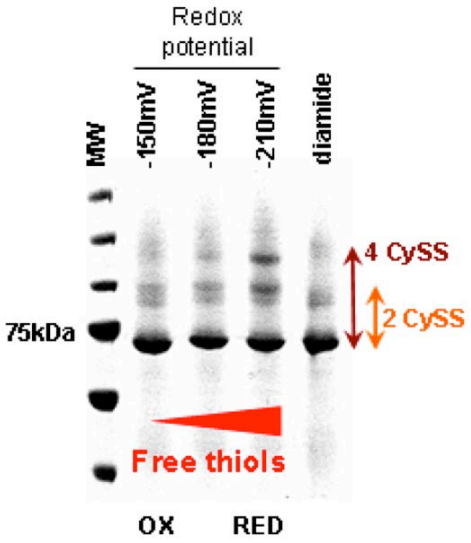
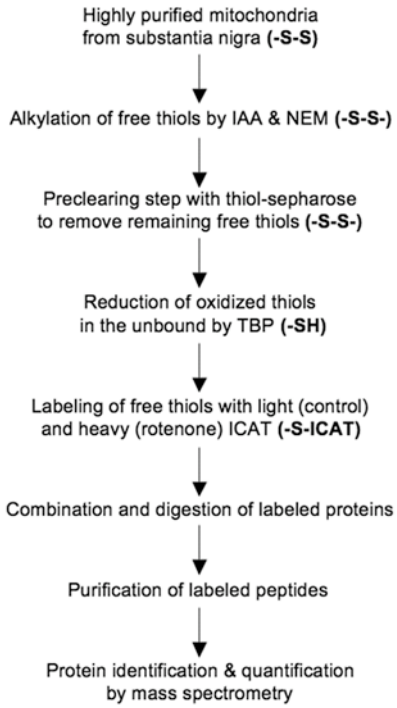
Supplemental figures



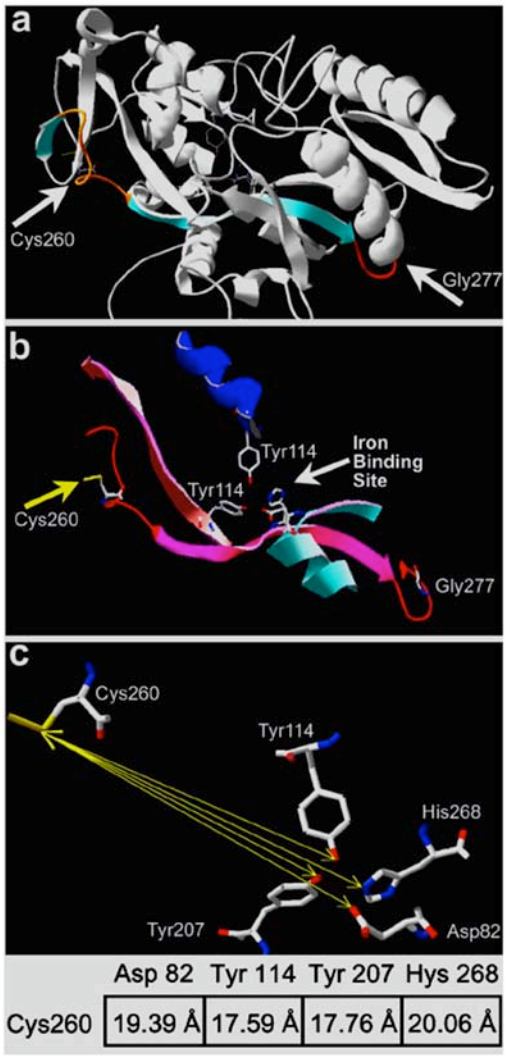
Supplemental figure 1. (a) Substantia nigra neurons of rotenone treated monkeys containing neuromelanin (brown) with iron deposition (blue). (b) Schematic of the approach used to label disulfides in histological specimens. (c) When two fluorophores with appropriate optical properties are in close proximity, targeting the same molecule, FRET occurs from the donor to the acceptor. We used FRET to determine proximity between the probe labeling oxidized thiols and a fluorescent primary antibody specific for Tf. Occurrence of FRET indicates thiol oxidation in Tf.



Supplemental figure 2. Tf contains redox sensitive thiols. After incubating purified Tf in buffers with known redox potential, free thiols were labeled with a 10kDa derivative of maleimide. Therefore, each free thiol will increase the appearent molecular weight of Tf by 10kDa, each disulfide (CySS) by 20kDa. Incubation of Tf in a reducing environment (i.e. -240mV) indicates that the protein has at least 4 redox sensitive thiols. As expected, incubation with diamide, which is a strong oxidant, induces thiol oxidation.

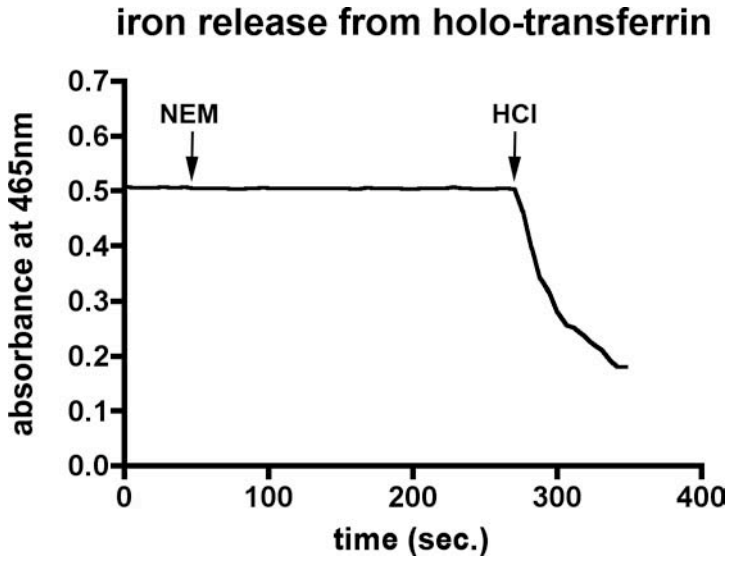


Supplemental figure 3. Schematic of the strategy used to labed oxidized proteins with the ICAT. ICAT is a biotinylated reagent bearing a functional group – iodoacetamide – that specifically reacts with thiol groups of cysteines. The ICAT molecule can be obtained in two different isotopic forms, heavy and light, which can be distinguished in the mass spectrometer. Therefore, two different samples can be labeled separately with the light and heavy ICAT reagents; the samples can therefore be pooled and analyzed in the same mass spectrometry run. The intensity of the peaks of the labeled peptides will provide a measure of their levels. We used the ICAT reagents to identify and quantify peptides containing oxidized thiols following rotenone treatment. Oxidized thiols (disulfides) were selectively modified with the ICAT reagents according to the procedure shown in the schematic in the figure and described in details in the methods.

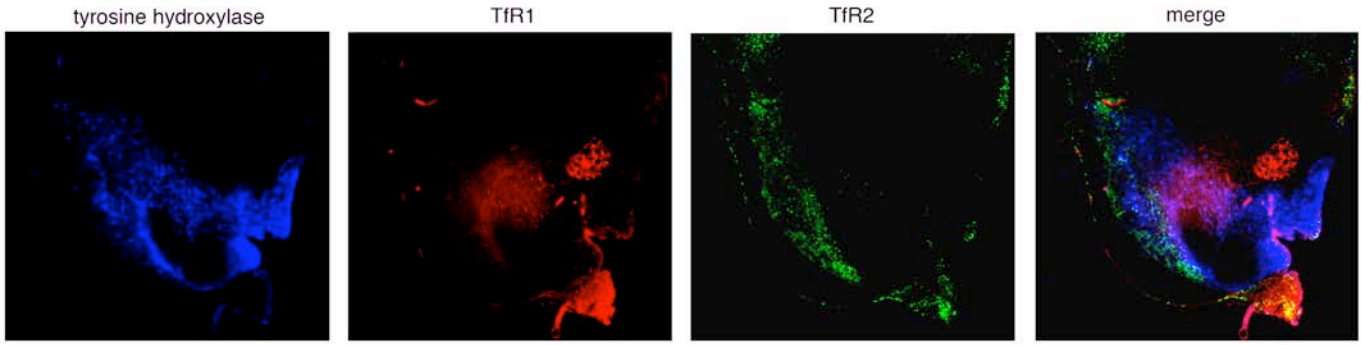


Supplemental figure 4. The oxidized residue found in rotenone treated animals (Cys260) is located on a loop flanking a strand that contains the histidine involved in the coordination of iron, His268. The distance between the oxidized sulfur atom and the coordination site for iron (figure 4c) raises the possibility that this electron might be accepted by Fe^{3+}, which would then be reduced to Fe^{2+} (1), with a subsequent drastic decrease in binding affinity ($K_d=10^{20}$ for Fe^{3+}, $K_d=10^{3}$ for Fe^{2+}) (2). Additionally, this residue is relatively exposed to the surface of the protein and the thiol group of Cys260 is oriented toward the surrounding environment rather than towards the interior of the

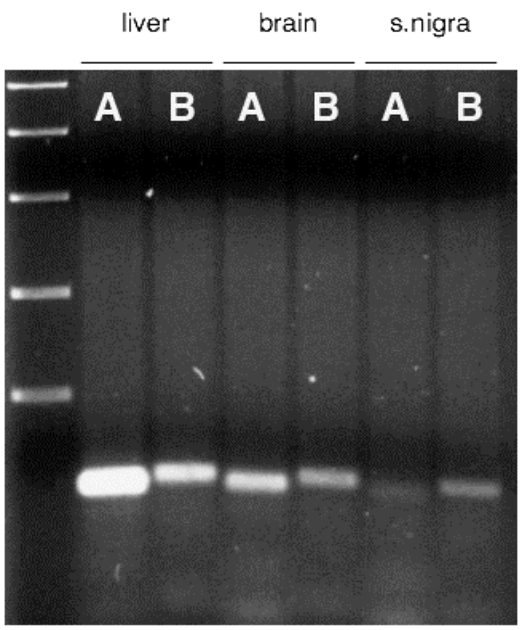
protein. This particular structural feature might render the residue particularly sensitive to the surrounding environment. Interestingly, the strand containing His268 is flanked by another loop containing a glycine residue, Gly 277, mutation of which (G277S) is associated significantly with PD (3) (G258S according to a different nomenclature in another paper (4)).



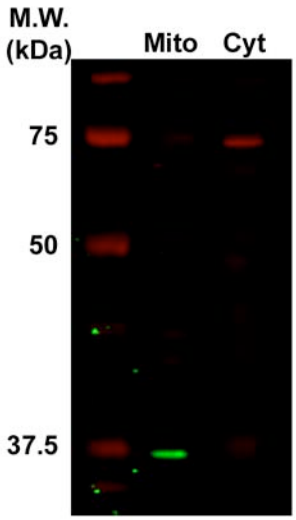
Supplemental figure 5. Alkylation of transferrin free thiols with NEM does not induce iron release *per se*. Iron release (ferric and ferrous) was monitored following the decrease of absorbance at 465nm. Total iron release was induced by acidification of the solution with HCl.



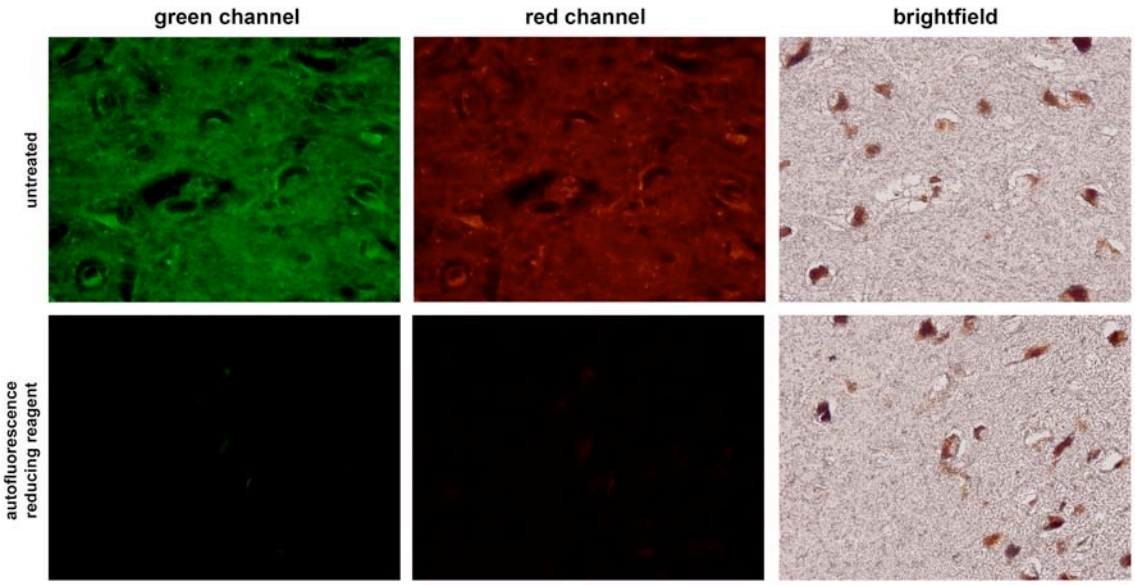
Supplemental figure 6. Immunofluorescent stain of a rat brain section to analyze the distribition of TfR1 and TfR2 in ventral midbrain. TfR2 (green) shows the highest co-localization with the dopaminergic neuron marker tyrosine hydroxylase.



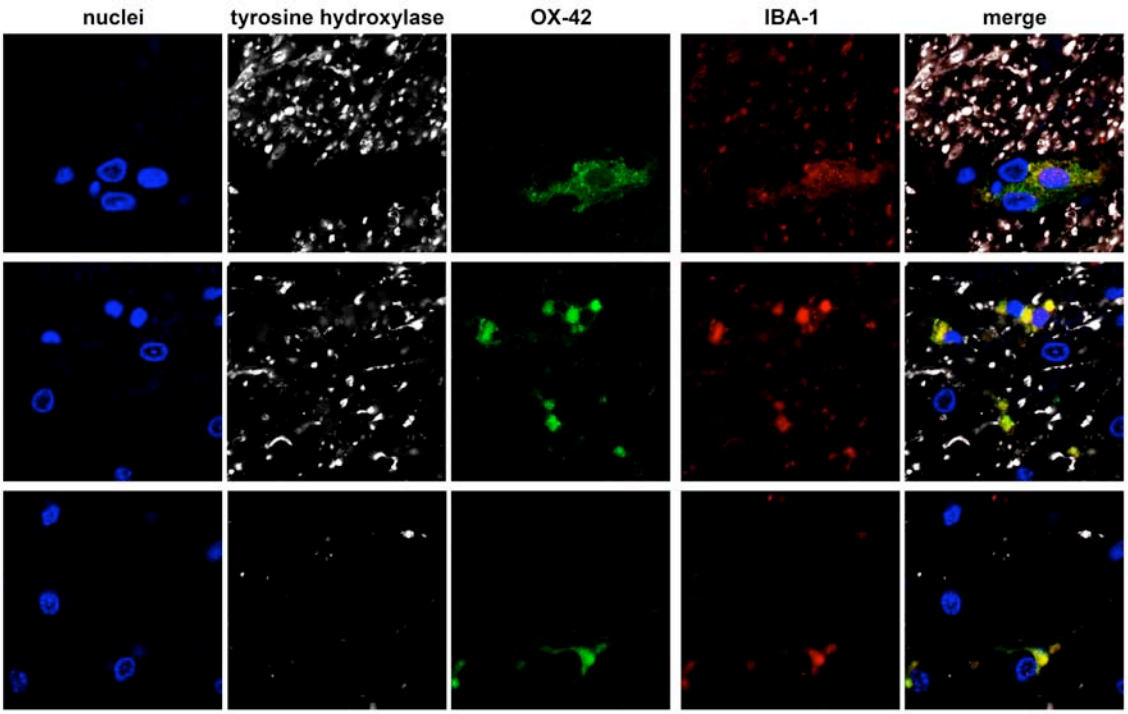
Supplemental figure 7. RT-PCR analysis to detect TfR2 mRNA from three human libraries. Amplification was performed using two different sets of primers (A and B), both of which indicate the presence of TfR2 mRNA in brain and, in particular, in the substantia nigra.



Supplemental figure 8. Western blot analysis to confirm the purity of the isolated mitochondrial fraction. Following sub-fractionation, 10 micrograms of protein were separated through SDS-electrophoresis. Hsp 70 was used as a cytosolic marker; the 39kDa subunit of the respiratory complex was used as a mitochondrial marker.



Supplemental figure 9. Treatment of human tissues that were incubated only with secondary antibodies with the autofluorescence-eliminating reagent results in effective reduction of non-specific signal in the green as well as in the red channel.



Supplemental figure 10. The microglial markers OX-42 and IBA-1 provide comparable signal.

Supplemental figures references

1. Boxer, S.G. 1990. Mechanisms of long-distance electron transfer in proteins: lessons from photosynthetic reaction centers. *Annu Rev Biophys Biophys Chem* 19:267-299.
2. Baker, H.M., Anderson, B.F., and Baker, E.N. 2003. Dealing with iron: common structural principles in proteins that transport iron and heme. *Proc Natl Acad Sci U S A* 100:3579-3583.
3. Borie, C., Gasparini, F., Verpillat, P., Bonnet, A.M., Agid, Y., Hetet, G., Brice, A., Durr, A., and Grandchamp, B. 2002. Association study between iron-related genes polymorphisms and Parkinson's disease. *J Neurol* 249:801-804.
4. Hershberger, C.L., Larson, J.L., Arnold, B., Rosteck, P.R., Jr., Williams, P., DeHoff, B., Dunn, P., O'Neal, K.L., Riemen, M.W., Tice, P.A., et al. 1991. A cloned gene for human transferrin. *Ann N Y Acad Sci* 646:140-154.