# Cyclic Peptide-Based Redox-Active Model of Rubredoxin

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## Supporting Information

**Peptide synthesis:** The linear precursor Alloc-Lys(Ac-Thr(tBu)-Cys(Trt)-Pro-Lys(Boc)-Cys(Trt)-Gly-Asn(Trt)-Glu(OtBu)-Lys(Boc)-Gly)-Lys(Boc)-Cys(Trt)-Gly-Trp(Boc)-$^{D}$Pro-Pro-Cys(Trt)-O-2Cl-Trt was assembled manually by solid-phase peptide synthesis on 2-chlorotrityl chloride resin using Fmoc chemistry with PyBOP/DIEA coupling. The Alloc protecting group was removed by reacting the beads with $Pd(PPh_3)_4$ (0.4 eq.) and $PhSiH_3$ (25 eq.) in dry DCM under argon for 1 hour. The reaction was repeated once. Then the beads were washed with DMF (×4), DMF/$H_2O$ 99:1, DMF (×2), DMF/DIEA 200:1, DMF (×2), DMF/sodium diethyldithiocarbamate 200:1 (w/w), DMF (×2), DCM (×2). The peptide was cleaved from the resin by treatments with DCM/TFA 99:1 for 2 minutes (×4). Each washing was neutralized with pyridine/MeOH 1:5 (5 mL). The combined washings were concentrated under reduced pressure and precipitated with petroleum ether. The solid was dissolved in DCM and pyridinium salts were extracted with water. The organic layer was dried over $Na_2SO_4$, filtered and evaporated to dryness. Cyclization was performed in dilute DCM solution (0.25 mM) with PyBOP (3 eq.)/DIEA (6 eq.) coupling. Removal of side chains protecting groups was performed in a TFA/TIS/$H_2O$/DDT (18 mL:0.6 mL:0.6 mL:600 mg) mixture. After 1.5 hours on stirring, the solution was evaporated to yield yellow oil which was precipitated with $Et_2O$. The crude peptide was purified by RP-HPLC (PurospherStar RP18e 5 µm C18 particles, 50 mm × 25 mm, solvent A = $H_2O$/TFA 99.9:0.1, solvent B = $CH_3CN/H_2O$/TFA 90:10:0.1, flow rate 30 mL/min, gradient 5 to 70% B in 28 minutes) to yield $L_{ZR}$ as a white powder after freeze-drying. Analytical RP-HPLC (PurospherStar RP18e 5 µm C18 particle, 150 mm × 4.6 mm, gradient 5 to 100% B in 18 minutes) were performed at 1.0 mL/min with UV monitoring at 214 nm. $t_R$(analytical) = 11.26 min. ESI-MS: m/z calculated: 980.44 $[M+2H^+]^{2+}$, 653.96 $[M+3H^+]^{3+}$, found: 980.5(2) $[M+2H^+]^{2+}$, 654.3(2) $[M+3H^+]^{3+}$.

**UV-Vis and CD titrations:** UV-Vis spectra were recorded on a Perkin-Elmer Lambda 35 spectrophotometer. CD spectra were recorded on an Applied Photophysics Chirascan spectropolarimeter or on a Biologic MOS-450 AF-CD spectropolarimeter. UV-Vis and CD spectrometers are equipped with a thermo-regulated cell holder. All buffer or metal solutions were prepared with MilliQ water (Millipore) and purged with argon. Buffer solutions were treated with Chelex 100 resin (Biorad) to remove metal traces. $Fe^{2+}$ titrations were performed with $Fe(SO_4)_2(NH_4)_2 \cdot 6H_2O$ under inert atmosphere in rubber-sealed quartz cells as previously described,[1, 2] except that all solutions were prepared in a glovebox (MBRAUN) under argon atmosphere. Additions of metal ion solutions in the cell were also performed in the glovebox. $Fe^{2+}$ apparent binding constants were measured by competition experiments with HEDTA in 100 mM HEPES buffer pH 7.0 as previously described for $Zn^{2+}$ but with UV-Vis monitoring of the LMCT bands around 320 nm.[1, 2] The $K_{app}$ value of HEDTA for $Fe^{2+}$ was calculated using published[3] values of $pK_a$ and log $\beta_{11}(Fe^{2+})$ for HEDTA ($pK_a$ = 1.60, 2.61, 5.38, 9.87; log $\beta_{11}$ = 12.2). All protonation constants were corrected upward by 0.11 to account for 0.1 M ionic strength as stated by Martell and Smith.[3, 4]

**Mössbauer spectroscopy:** $^{57}Fe$ Mössbauer spectra have been recorded at 4.2 K either on a low field Mössbauer spectrometer equipped with a Janis SVT-400 cryostat or on a strong-field Mössbauer spectrometer equipped with an Oxford Instruments Spectromag 4000 cryostat containing an 8 T split-pair superconducting magnet. Both spectrometers were operated in a constant acceleration mode in transmission geometry. The isomer shifts are referenced against that of iron metal at room temperature. The analysis of the data was performed with the software package WMOSS (WEB Research, Edina, MN, www.wmoss.org). The $^{57}Fe^{II} \cdot L_{ZR}$ (1.17 mM, HEPES 20 mM pH 7.5) sample was prepared in the glovebox by mixing 0.9 equiv. of $^{57}Fe(SO_4)_2(NH_4)_2 \cdot 6H_2O$ with $L_{ZR}$ and adjusting the pH to 7.5. For the Mössbauer measurements of the oxidized form, a $^{57}Fe^{II} \cdot L_{ZR}$ sample (1.72 mM, HEPES 100 mM pH 7.55, TCEP 17.2 mM) was prepared and exposed to air. The samples were then frozen into liquid nitrogen.

**EPR spectroscopy:** Spectra were recorded on a Bruker EMX (9.5 GHz) EPR spectrometer equipped with an ESR 900 helium flow cryostat (Oxford Instruments) between 4.9 and 20 K under non-saturating conditions. No resonances were detected above 250 mT.

**Electrochemistry.** Electrochemistry was performed in an anaerobic chamber (Jacomex, France) with a residual concentration of $O_2$ below 1.5 ppm, using an Autolab PGSTAT 128N potentiostat with a standard 3-cell electrode, using a platinum grid as counter electrode and a saturated calomel electrode as reference electrode. The working electrode was a gold disc (diameter 1 mm) that we cleaned by repetitively cycling in a 0.1 M $H_2SO_4$ aqueous solution

and then left to incubate overnight in a 1 mM ethanolic solution of *p*-mercaptobenzoic acid. We checked that the intensity of the oxidation wave is proportional to the square root of the scan rate, thereby showing that we address species that diffuse freely in solution (Fig. S2). We determined a diffusion coefficient of about 1.1 $10^{-6}$ $cm^2s^{-1}$ for the $L_{ZR}$ complex, comparable with that found for small proteins such as cytochrome $c_6$.[5]

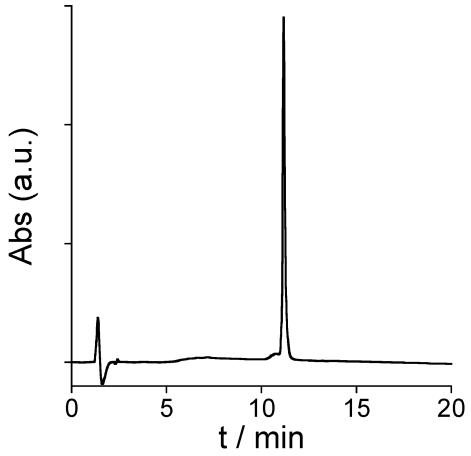


**Fig. S1** Analytical HPLC chromatogram of $L_{ZR}$.

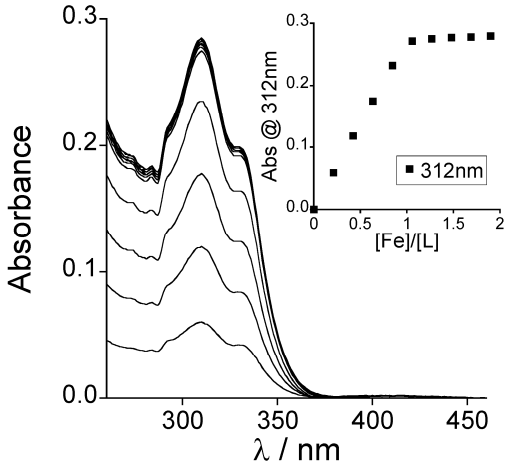


**Fig. S2** UV-Vis titration of $L_{ZR}$ 44 µM by $Fe^{2+}$, HEPES buffer 20 mM pH 7.5, TCEP 250 µM, 298 K (path length = 1.0 cm). All spectra were corrected for dilution and the spectrum of the *apo*-peptide was subtracted to each spectrum.

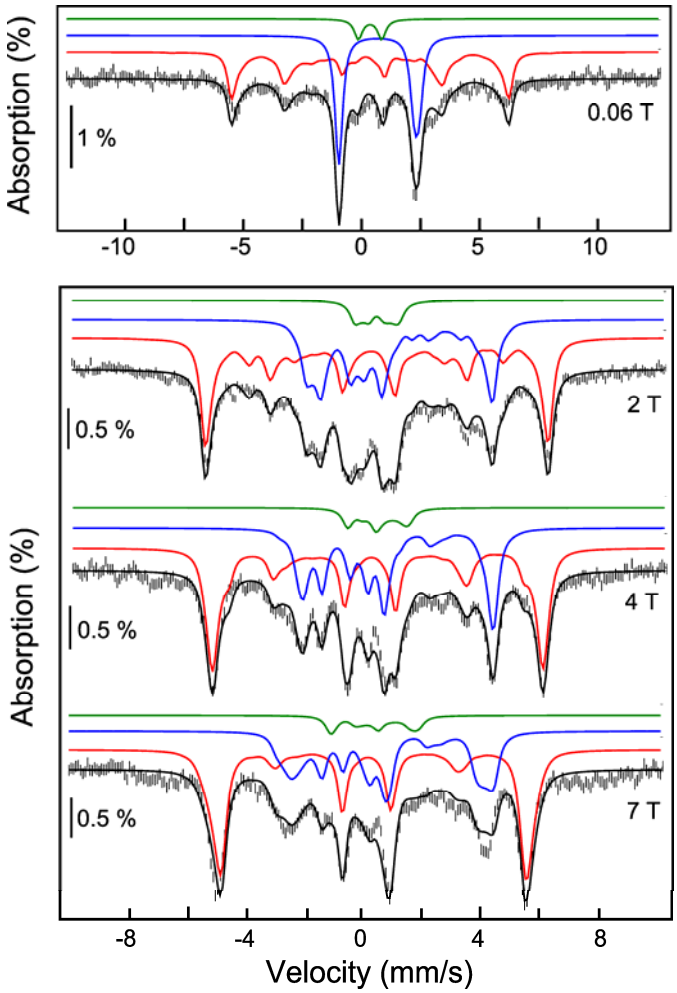


**Fig. S3** Mössbauer spectra (vertical bar) of an $^{57}Fe$ solution of $Fe^{II}{\cdot}L_{ZR}$ in HEPES buffer after exposition to air in the presence of TCEP. Spectra were recorded at 4.2 K with magnetic fields ranging from 0.06 T to 7 T applied parallel to the γ-beam. Spectra can be deconvoluted in 3 components: $Fe^{II}{\cdot}L_{ZR}$ component (blue simulation line) accounting for 41 % of total intensity, $Fe^{III}{\cdot}L_{ZR}$ (red simulation line) component accounting for 51 % of total intensity, and an impurity accounting for 6 % of total intensity. Mössbauer parameters of this third component (S=0 ground state with isomer shift of 0.37 $mm.s^{-1}$ and quadrupolar splitting of 0.97 $mm.s^{-1}$) are in agreement with an iron-oxo species.

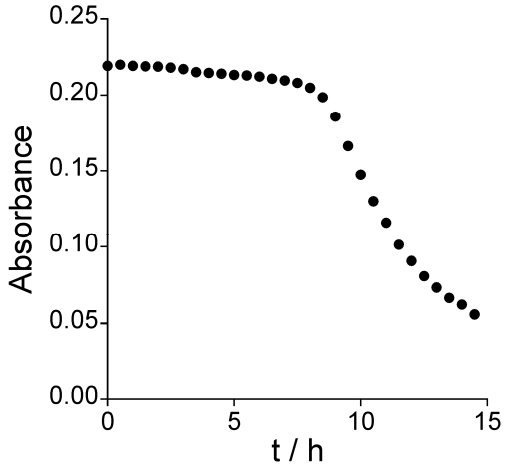


**Fig. S4** Absorbance (491 nm) monitoring of the stability of $Fe^{III}{\cdot}L_{ZR}$ (42 µM) under air in HEPES buffer 20 mM pH 7.5, TCEP 750 µM, 298 K (path length = 1.0 cm). The absorbance was recorded every 30 minutes over 50 hours after air oxidation of $Fe^{II}{\cdot}L_{ZR}$.

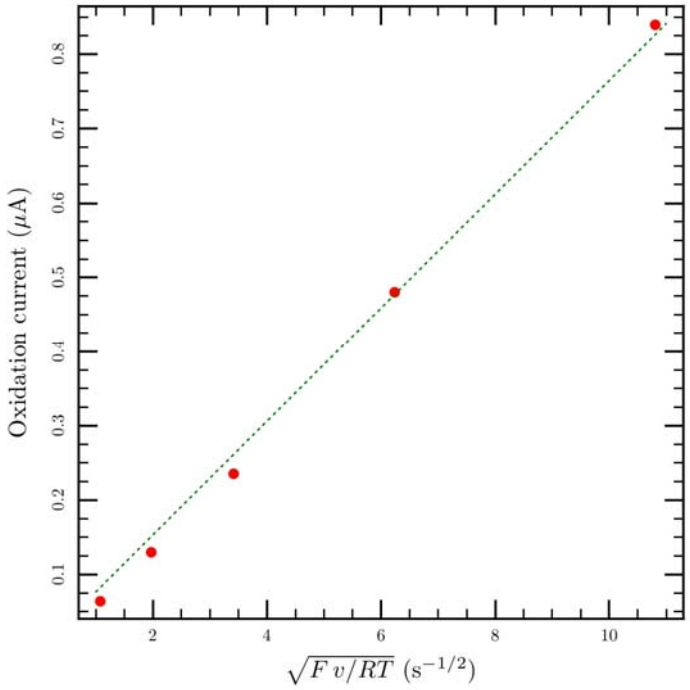


**Fig. S5** Plot of the peak current for the oxidation peak as a function of the square root of Fv/RT. For a purely diffusive system, this is expected to yield a straight line whose equation is given by $i = F\ p\ r^2\ C\ (D\ F\ v/RT)^{1/2}\ \beta$ where $r$ is the radius of the electrode disc, $F$ the faraday constant, $D$ the diffusion coefficient of the complex, $C$ the concentration of the complex, $v$ the scan rate, and β is either 0.4463 or 0.351 depending on whether the electrochemical system is fully reversible or only quasi-reversible.[6]

## References.

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