Supporting information for
Oxygen Reactivity of the Biferrous Site in the de novo Designed Four Helix Bundle Peptide DFsc: Nature of the "Intermediate" and Reaction Mechanism

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Materials and Methods

Sample Preparation. MOPS (3-(N-morpholino)propanesulfonic acid) buffer (Sigma), sodium chloride (Sigma), ferrous ammonium sulfate hexahydrate (Mallinckrodt), and sodium dithionite (Sigma) were commercially purchased and used as obtained. Buffers were degassed by freeze pump thaw cycles with 99.9% pure argon when appropriate. All manipulations were done in the inert atmosphere of a glove box. DFsc and variants were expressed and purified as previously reported[12]. However, the cells were broken using a modified freeze thaw method, where 24 mls of water was added to 1 L of broken cells in the final lysis step. Cells were centrifuged at 6,000 rpm for 15 mins. The supernatant was collected and lyophilized. Following lyophilization, 3.5 ml of water was added to each liter of lyophilized crude protein. A 500 µl sample was removed for HPLC: 3 ml of buffer A (99.9% water, 0.1%TFA) and 800 µl of buffer B (90% acetonitrile, 10% water, 0.1% TFA) were added to the sample. The precipitated proteins were removed by centrifugation followed by filtration. The sample was loaded onto a Vydac C4 column with a linear gradient from 40% B to 60% B over 40 min. All DFsc proteins eluted at approximately 53%B. The molecular weight was confirmed by MALDI and SDS-PAGE. Protein concentrations for experiments were determined by using a molar extinction coefficient of 8250 $M^{-1}cm^{-1}$ at 280 nm.

UV/vis and Kinetic measurements. All reactions were performed at 25 ^{o}C. Fe(II) in 150 mM MOPS, 150 mM NaCl pH 7.0. Protein concentrations were 100 µM and 200 µM ferrous iron was added to the protein solution, mixed and allowed to equilibrate for 30min prior to loading into the Stop-flow. UV-visible absorption spectra were obtained using an Agilent 8453 diode array spectrophotometer. Stopped-flow absorption kinetics with ~2 ms dead time were obtained using an Applied Photophysics SX.18MV stopped-flow absorption spectrophotometer equipped with a Hg/Xe arc lamp and outfitted with PEEK tubing. The tubing, plungers, and valves of the stopped-flow instrument were made anaerobic by washing with dithionite solution followed by several washes with degassed buffer. O_2 inclusion was prevented by a stream of N_2 gas through the system. The cell path length was 1 cm and temperature was maintained using a temperature bath. Fitting of the kinetic data was performed in Origin in order to get a first approximation to kinetic values. The data were fit to a 1st order exponential function of the form $A = A_0 e^{-kt} + A_1$, where A is the measured absorbance at 520nm, A_0 the pre-exponential factor, k the extracted rate constant and A_1 accounts for non zero absorbance at t = 0. For the formation phase fits included data up to 4s for DFsc and 1s for DFscE11D. Kinetic simulations to the whole data set were performed using Berkeley Madonna. Initially, an A → B → C (where B represented the 520nm species and C was allowed to have an extinction coefficient greater than 0 to account of the precipitation known to occur at long times) model was used but found not to accurately reproduce the data, Figure S2. A model with three phases (A → B → C → D, where B and C have the same extinction coefficient at 520 nm), however fits and gives a reasonable molar extinction for the Fe^{III}←tyr CT. Rate constants quoted in the text are the average of simulations from 3 sets of independent data.

Resonance Raman. Resonance Raman (rR) spectra were obtained using a Princeton Instruments ST-135 back-illuminated CCD detector on a Spex 1877 CP triple monochromator with 1200, 1800, and 2400 grooves/mm holographic spectrograph gratings. Excitation was provided by Coherent I90C-K Kr^+ and Innova Sabre 25/7 Ar^+ CW ion lasers at ~10mW at the sample. Spectral resolution was <2 cm^{-1}. The samples were prepared as in the kinetic study and flash frozen with liquid nitrogen in 5 mm NMR tubes at the time of maximum 520nm species concentration. The samples were cooled to 77 K in a quartz liquid N_2 EPR finger Dewar (Wilmad) and hand spun during scan collection to minimize sample decomposition. Isotopic substitution experiments were performed with $^{18}O_2$ (ICON, 99% labeled) and $H_2{}^{18}O$ (Cambridge Isotopes).

Magnetic Circular Dichroism. UV-visible magnetic circular dichroism (MCD), at 2 K, were measured with a Jasco J-810-150S spectrapolarimeter coupled with an S-20 photomultiplier tube and an Oxford SM4000-8T magnet. MCD spectra were measured using cells which were fitted with two quartz disks and a 3-mm rubber spacer. To eliminate zero-field baseline effects in MCD, the spectra are an average of the +7 T and -7 T scans, [7 - (-7)]/2 T.

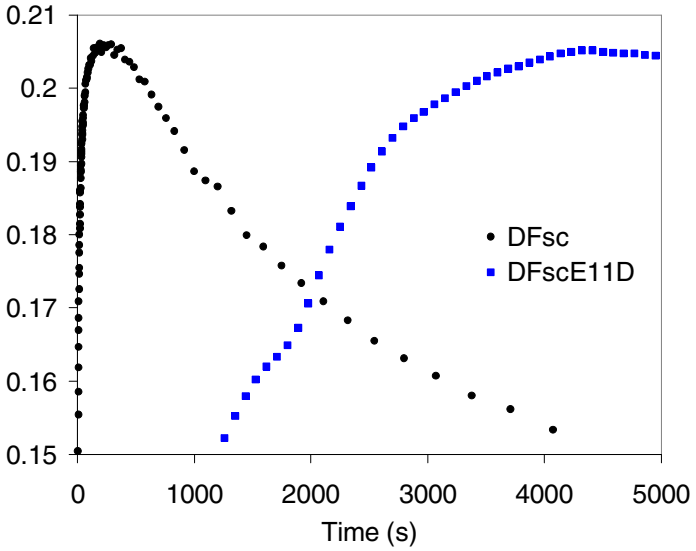

Figure S1. Time course of formation of the 520nm species under aerobic conditions. Reactions were initiated by adding Fe^{II} in acidic solution directly to the cuvette and monitored at 520nm.

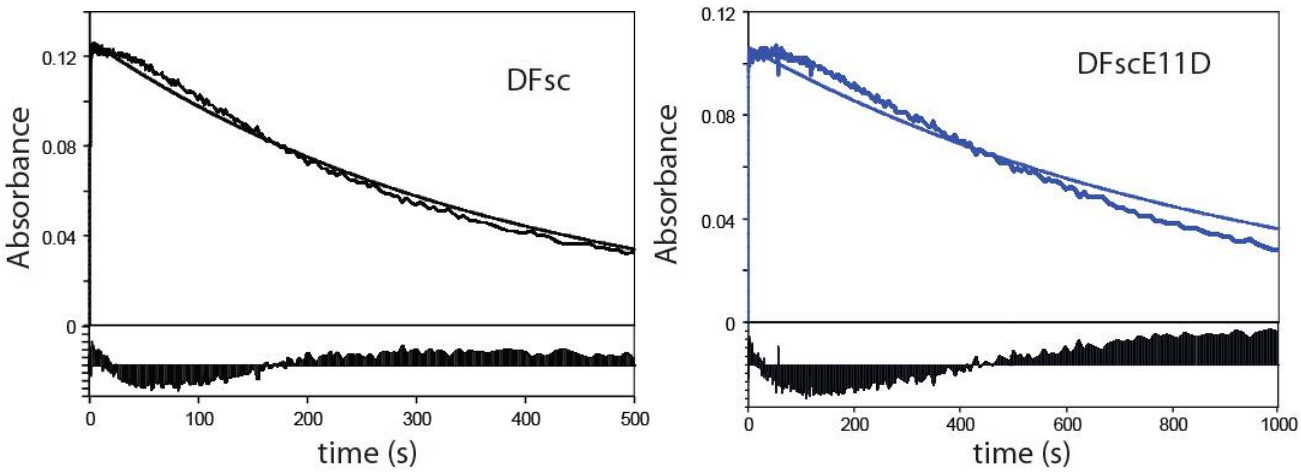

Figure S2. Kinetic traces of the O_2 reaction monitored at 520nm. Kinetic behavior (data points) was modeled (solid lines) with Berkeley Madonna to A → B → C, where both B and C were allowed to have absorbencies at 520nm. Residuals are shown in the bottom panels. Log scale time courses of the experimental data with the three phase fit are shown in the main text.

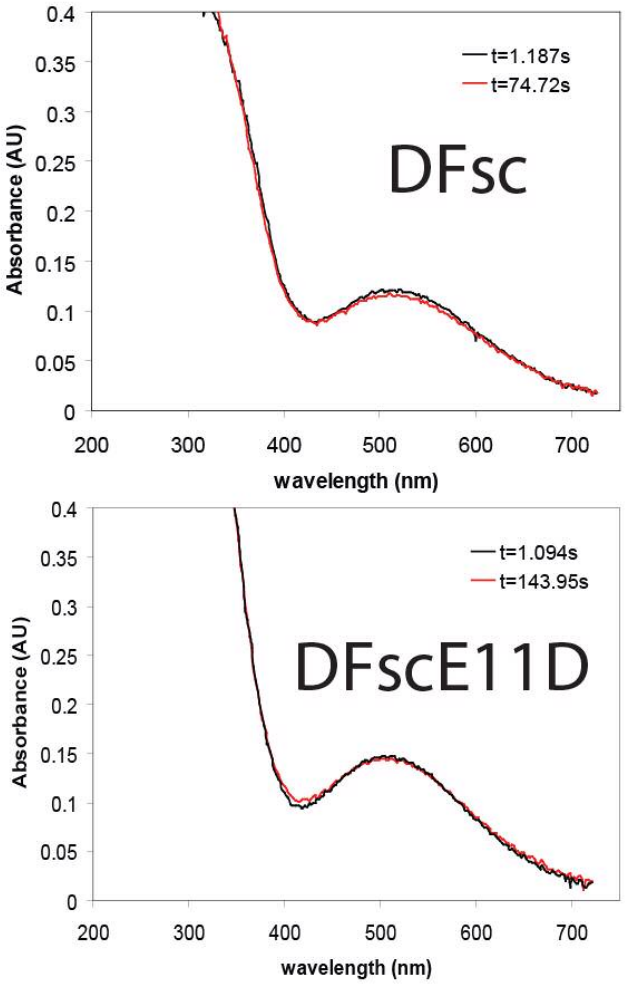

Figure S3. Overlays of the 520 nm species formed by reaction of the anaerobically Fe^{II}-preloaded DF peptides with O_2 saturated buffer at the times indicated in each plot.

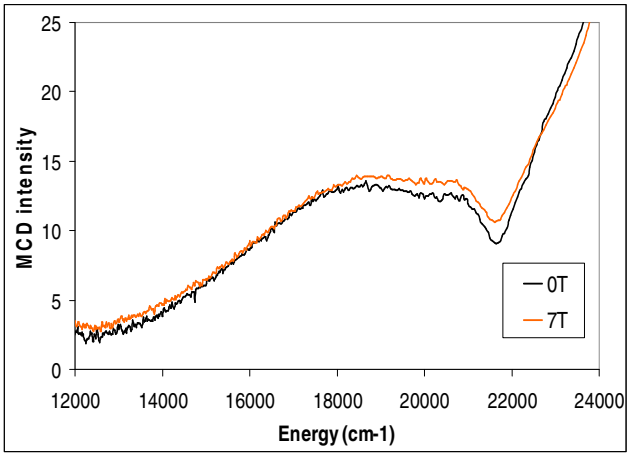

Figure S4. Low temperature CD and MCD spectra of 520nm species collected at 2K. 7T spectrum is the average of spectra collected at positive and negative fields.