

Supplementary Information

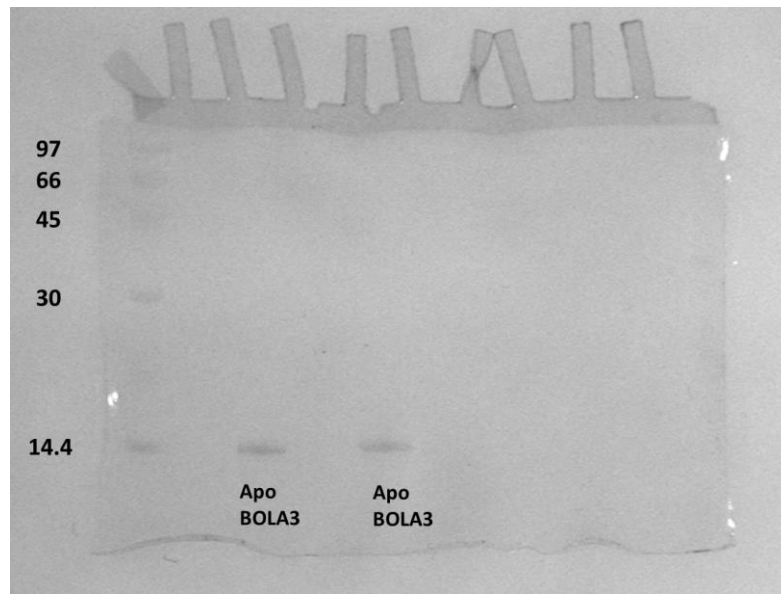


Figure S1. SDS-PAGE for Apo BOLA3 purified from Ni column.

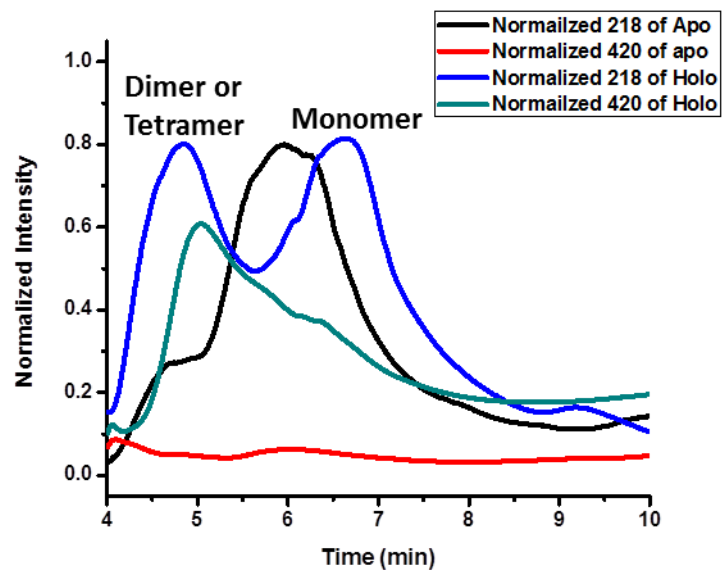


Figure S2. Size exclusion chromatography for oligomeric state determination of apo and holo BOLA3. HPLC traces after being smoothed using Graph Pad Prism software version 7.0, for apo and reconstituted BOLA3 with the intensities normalized between (0 and 1). The absorbance at 218 nm for reconstituted holo BOLA3 shows two distinct peaks, presumably one for the dimer or tetramer (holo) and one for monomer (residual apo), which also matches with the one major peak for apo. The absorbance at 218 nm for apo (black trace) and the 218 (blue trace) and 420 (green trace) traces for holo match at around 5 min confirming the dimeric/tetrameric state.

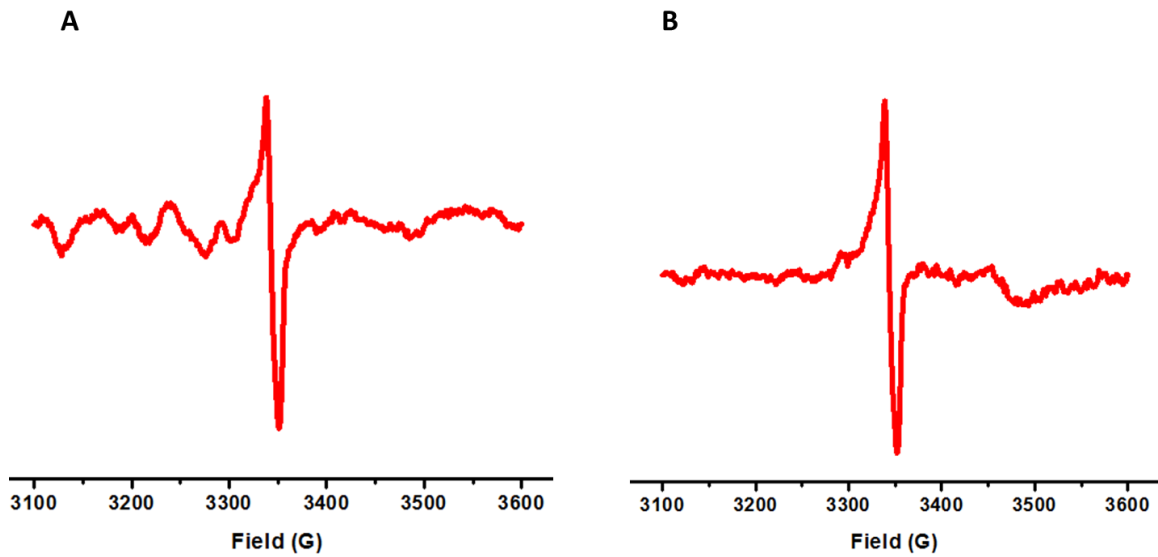


Figure S3. EPR spectrum of holo (A) C59A and (B) H96A variants of BOLA3 after dithionite reduction and rapid freezing.

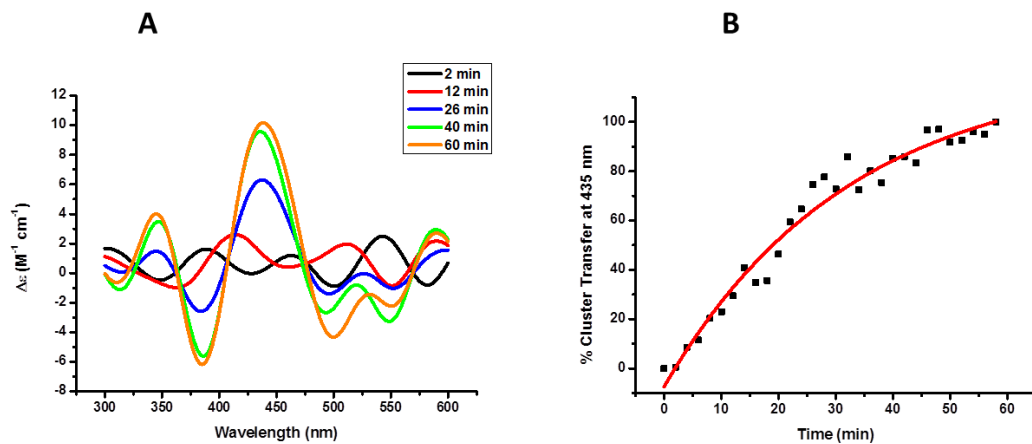


Figure S4. Cluster transfer from holo C59A BOLA3 to apo FDX 2 in 50 mM HEPES, 100 mM NaCl, pH 7.5. Spectra were recorded every 2 min after the addition of holo BOLA3, which was added to yield a final protein concentration ratio of 1:1, and converted to percent cluster transfer yielding a rate constant of $1820 \pm 220 \text{ M}^{-1} \text{ cm}^{-1}$

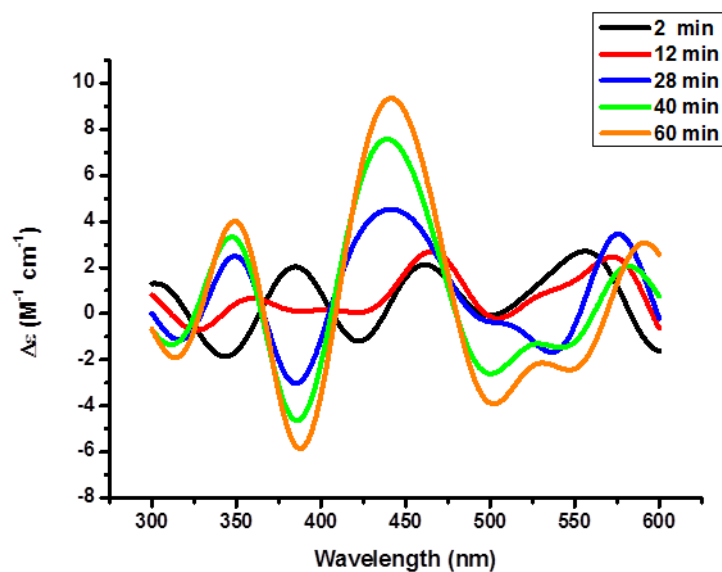


Figure S5. Cluster transfer from holo H96A BOLA3 to apo FDX 2 in 50 mM HEPES, 100 mM NaCl, pH 7.5. Spectra were recorded every 2 min after the addition of holo BOLA3, which was added to yield a final protein concentration ratio of 1:1.

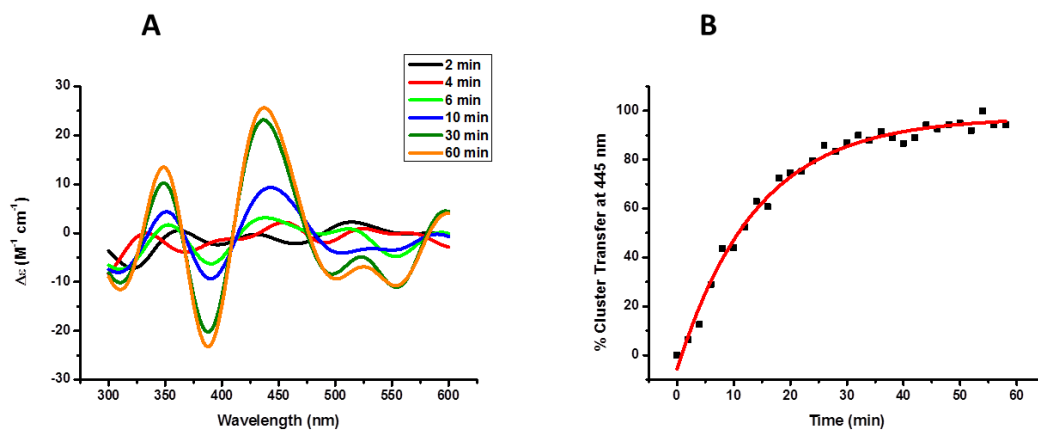


Figure S6. Cluster transfer from holo BOLA3, reconstituted without any DTT or thiol source, to apo ferredoxin 1 in 50 mM HEPES, 100 mM NaCl, pH 7.5. Spectra were recorded every 2 min after the addition of holo BOLA3, which was added to yield a final protein concentration ratio of 1:1, and converted to percent cluster transfer.

DTT Concentration (mM)	Rate Constant ($M^{-1} \text{ cm}^{-1}$)
5	2240 ± 430
2	1400 ± 240
0.5	2810 ± 660

Table S1. Table showing rate constants for cluster transfer from holo BOLA3 to apo FDX2 under varying DTT concentrations. The rates are roughly comparable, demonstrating that DTT does not influence reaction rates under our reaction conditions.