Supporting Information

Figure S1. Single turnover reactions under anaerobic conditions. When the absence of the initial enzyme-AHQQ complex at 532 nm occurs, it is a result of its low extinction coefficient. **A**. Active site variant H154N. Representative spectra at time points: 0.2, 0.6, 1, 1.0, 1.6, 3.2, 4.8, 6, 7, 9, 10.2, 11.6, 13, 15, 17.5, 20, 22.5, 26, 31.5, 37, and 42.5 min. **B**. Active site variant R179S. Representative spectra at time points: 0.2, 1, 1.8, 2.6, 4.2, 5.8, 7.4, 9.8, 12.2, 14.6, 20, 26, 32, 38, 46, 54, and 60 min.

Figure S2. Anaerobic to aerobic transitions. The 338 nm peak decreases as the 318 nm peak increases. H154N. Representative spectra at time points: 0.2, 0.6, 1.2, 1.8, 3, 4.2, 5.4, 6.6, 7.8, 9, 10.2, 11.4, 12.6, 13.8, 15, 18, 21, 24, 27, and 30 min.

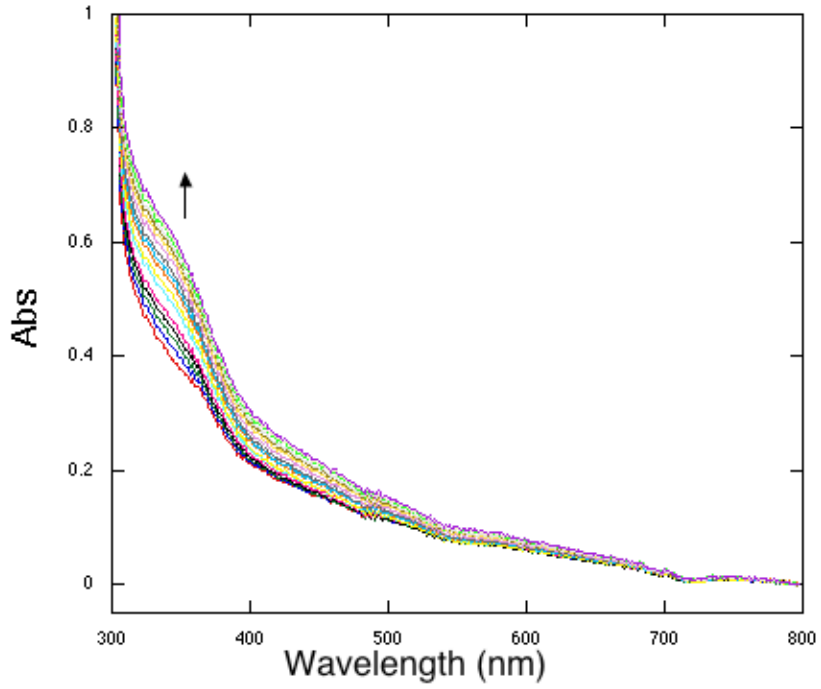
Figure S3. Single turnover reactions under aerobic conditions. **A**. H154N. Representative spectra at time points: 0, 0.6, 1.2, 1.8, 3, 4.2, 5.4, 6.6, 7.8, 9, 10.8, 12.6, 14.4, 18, 22.5, 28.5, 34.5, 39, 45 min. **B**. R179S. Representative spectra at time points: 0.2, 0.6, 1, 1.4, 2.6, 3.4, 4, 6, 8, 10, 12.2, 14.2, 19, 22.5, 27, 32, 37, and 42.5 min.

Figure S4. Binding of PQQ to mutant enzymes. **A.** H154N K_D. Binding curve of PQQ (20 nM) to H154N form of PqqC as determined by fluorescence spectroscopy. Data yielded a K_D of 0.66 nM. **B.** Y175S K_D. Binding curve of PQQ (20 nM) to Y175S form of PqqC as determined by fluorescence spectroscopy. Data yielded a K_D of 12.23 nM.

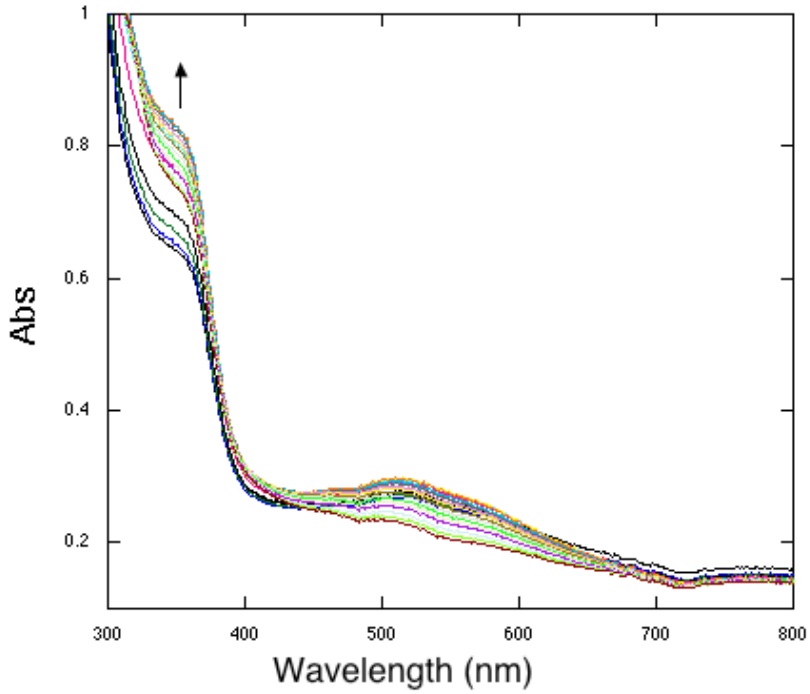
Figure S5. $PQQH_2$ reoxidation to PQQ in the presence of O_2 core mutants. **A**. H154N. **B**. Y175S.

Figure S1

A.


Abs
Wavelength (nm)
1
0.8
0.6
0.4
0.2
0
300
400
500
600
700
800

B.


Abs
Wavelength (nm)
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0.6
0.4
0.2
300
400
500
600
700
800

Figure S2

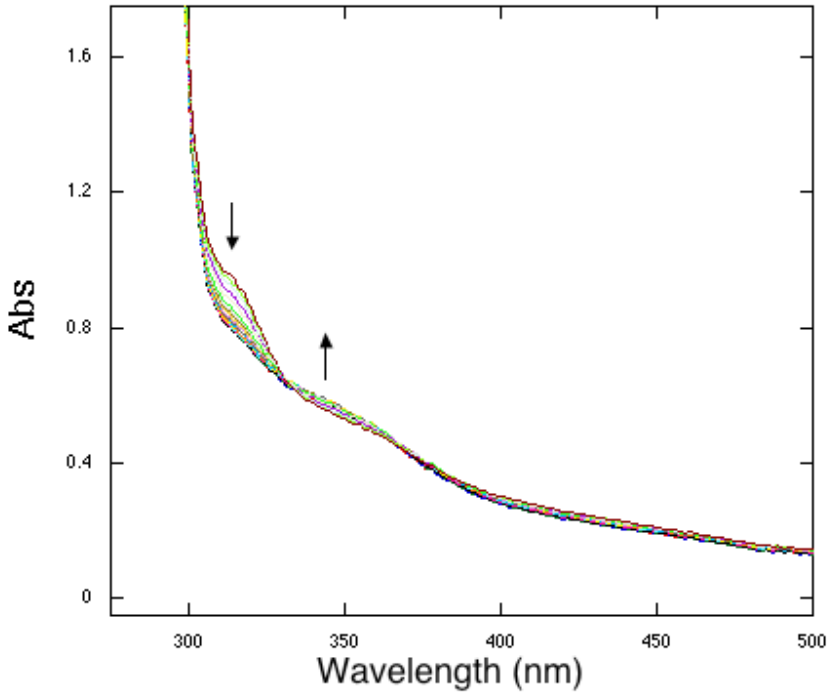
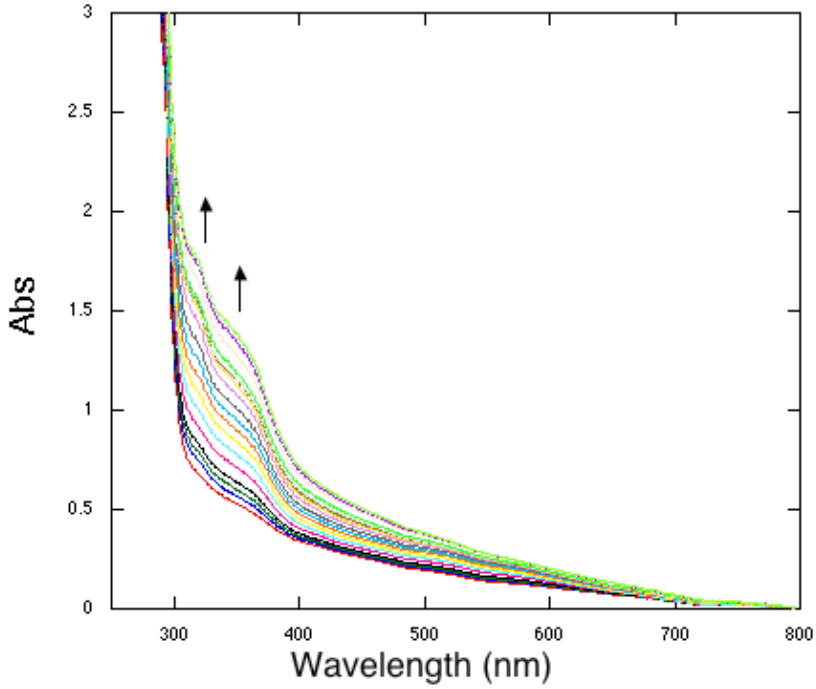


Figure S3

A.


Abs
Wavelength (nm)
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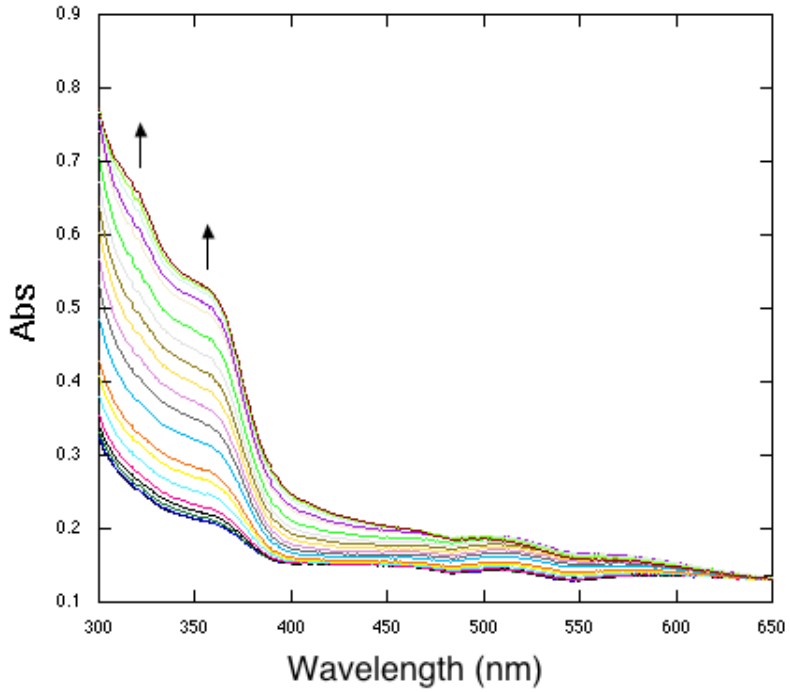
1
1.5
2
2.5
3
300
400
500
600
700
800

B.


Abs
Wavelength (nm)
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0.4
0.5
0.6
0.7
0.8
0.9
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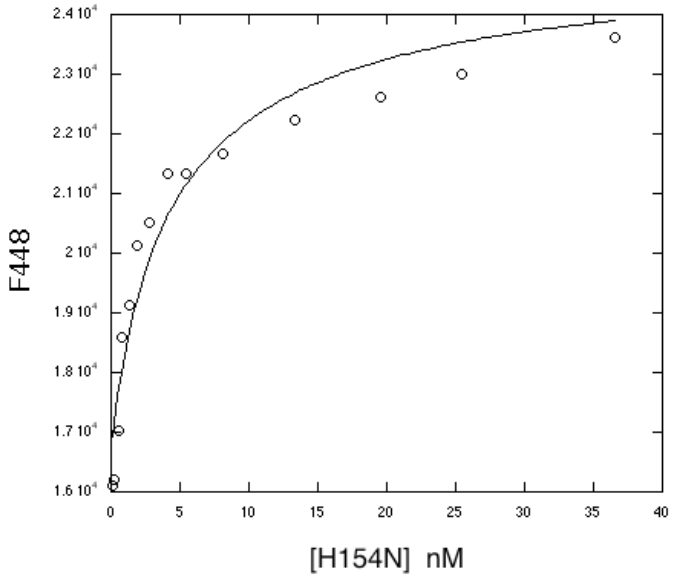
350
400
450
500
550
600
650

Figure S4

A.



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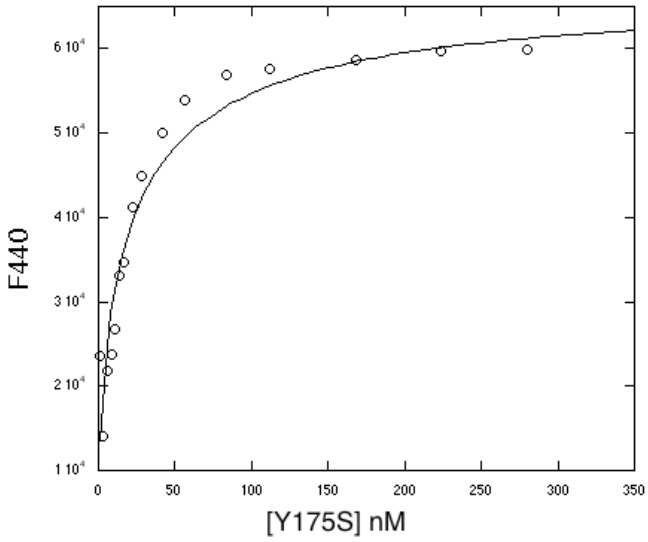
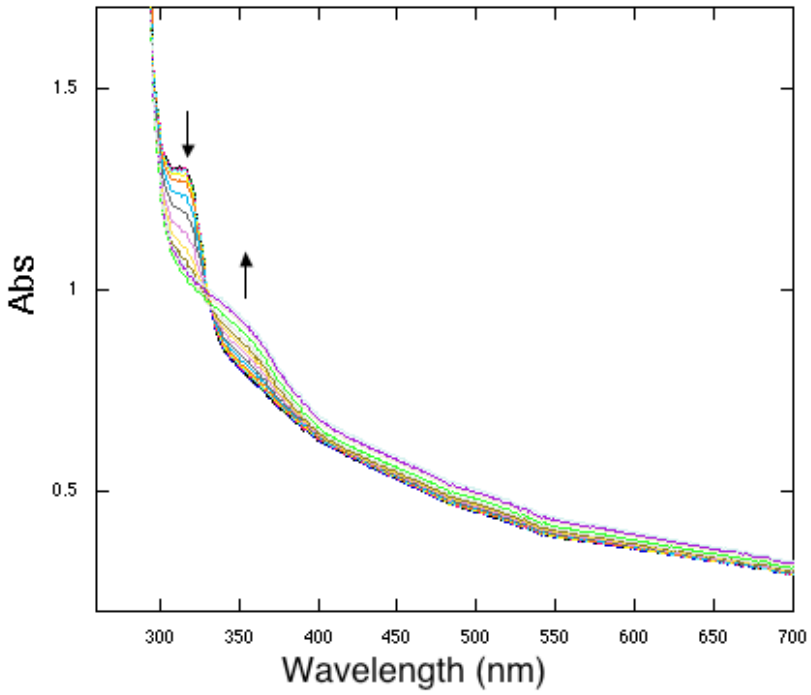


Figure S5

A.


Abs
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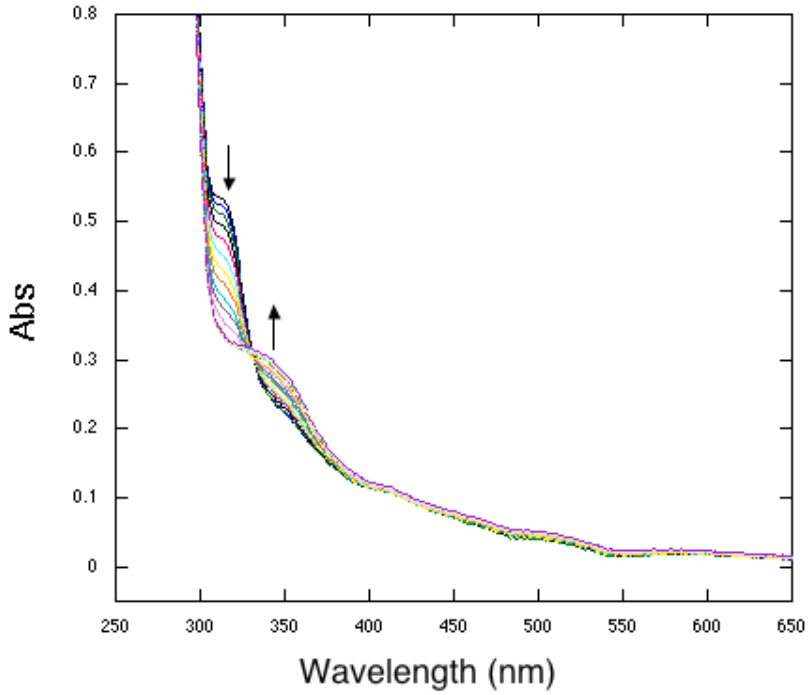
1
0.5
300
350
400
450
500
550
600
650
700
Wavelength (nm)

B.


Abs
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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
250
300
350
400
450
500
550
600
650
Wavelength (nm)