SUPPORTING INFORMATION

Structural Determinants of Flavin Dynamics in a Class B Monooxygenase

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Table S1. X-ray Diffraction Data Collection and Refinement Statistics

	Ligand-free	$NADP^+$
Space group	$P2_1$	$P2_1$
Unit cell parameters (Å,°)	a = 76.44, b =155.27, c = 88.27, β = 110.33	a = 80.23, b = 153.81, c = 89.87, β = 109.28
Resolution (Å)[a]	61.4 - 2.20 (2.24 - 2.20)	62.9 - 2.10 (2.14 - 2.10)
Observations	340416	401873
Unique refls.	96363	115533
$R_{merge}(I)$[a]	0.108 (1.235)	0.082 (0.445)
Mean I/σ[a]	10.8 (0.9)	9.4 (1.7)
$CC_{1/2}$[a]	0.995 (0.484)	0.993 (0.740)
Completeness (%)[a]	98.7 (89.2)	96.6 (95.9)
Multiplicity[a]	3.5 (2.8)	3.5 (2.9)
No. of atoms		
Protein	13919	13765
FAD	212	212
$NADP^+$	N/A	192
Water	387	571
R_{cryst}[a]	0.194 (0.334)	0.204 (0.205)
R_{free}[a,b]	0.230 (0.378)	0.241 (0.255)
rmsd bonds (Å)	0.007	0.008
rmsd angles (°)	0.942	0.986
Ramachandran plot[c]		
Favored (%)	96.80	97.41
Outliers (%)	0.11	0.00
Clashscore (PR)[c]	3.38 (99)	2.94 (99)
MolProbity score (PR)[c]	1.53 (98)	1.25 (100)
Average B (Å^2)		
Protein	35.1	28.8
FAD	27.5	23.4
$NADP^+$	N/A	25.5
Water	30.1	26.1
Coord. error (Å)[d]	0.30	0.22
PDB code	7JVK	7JVL

[a]Values for the outer resolution shell of data are given in parenthesis. [b]2% test set. [c]From MolProbity. The percentile ranks (PR) for Clashscore and MolProbity score are given in parentheses. [d]Maximum likelihood-based coordinate error estimate from PHENIX.

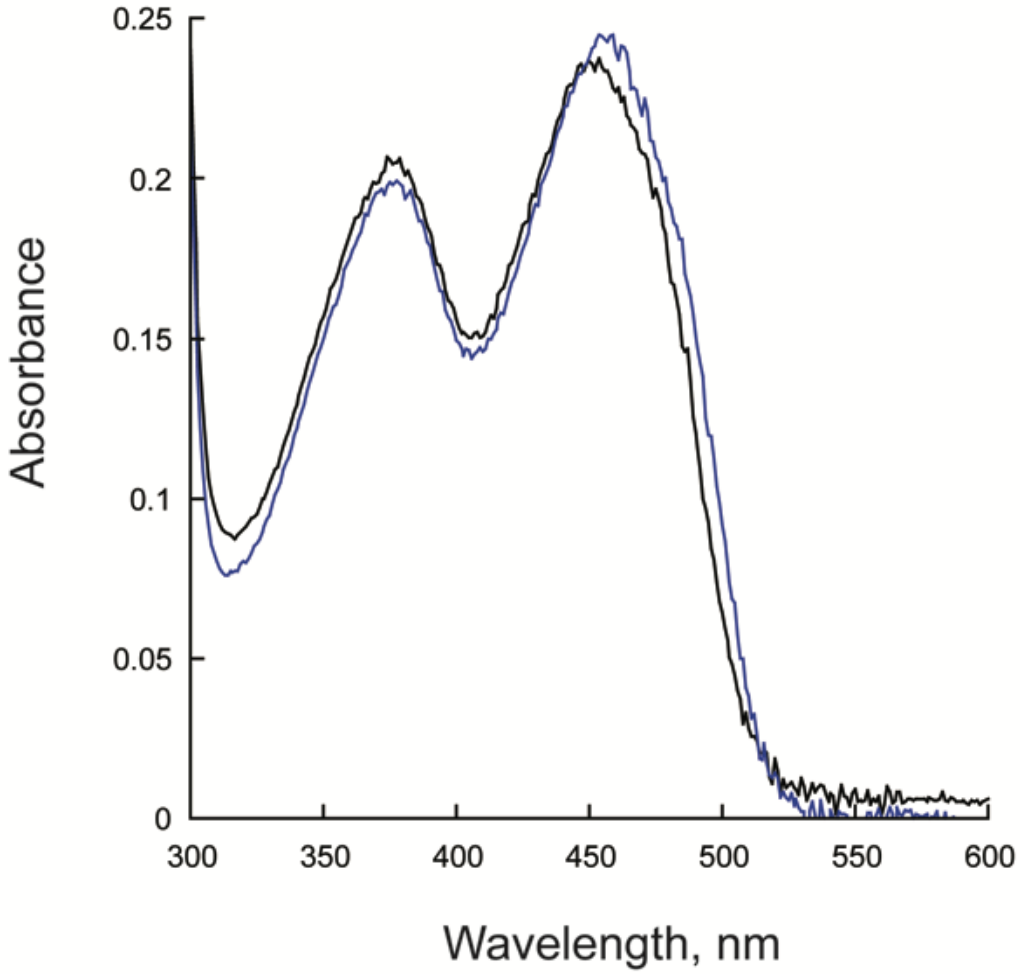

Figure S1. UV-visible spectra of SidA (black) and M101A (blue).

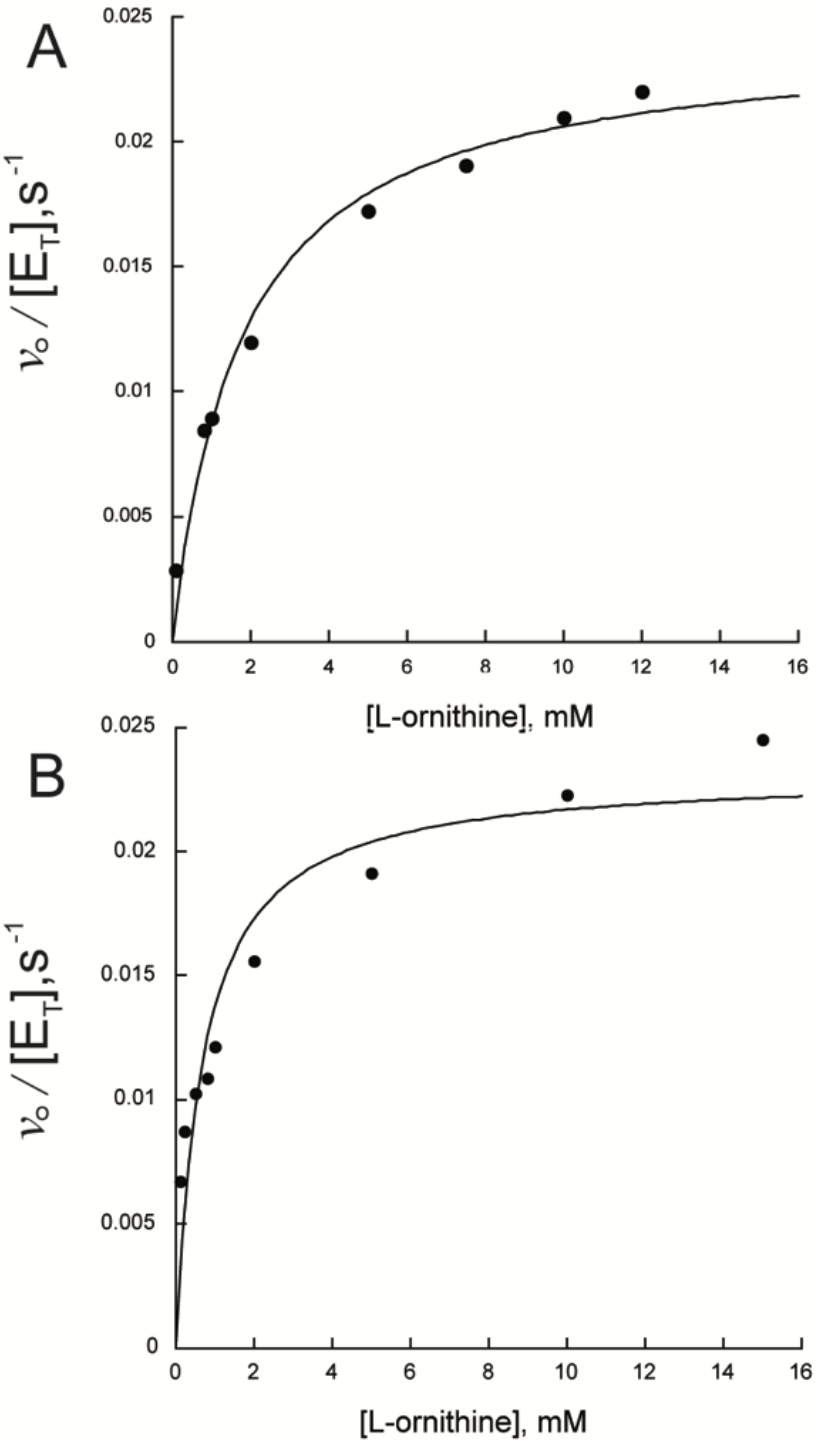

Figure S2. Activity of M101A under steady-state conditions. (A) Results from the L-Orn hydroxylation. (B) Results from oxygen consumption assays.

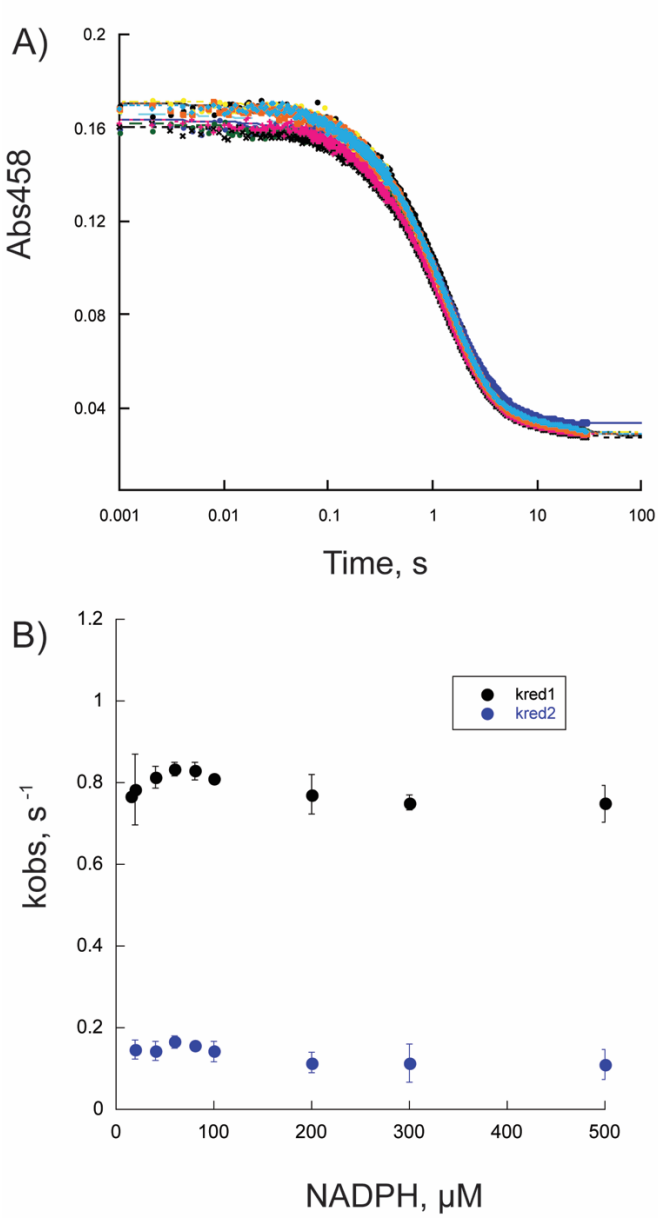

Figure S3. Reduction of the FAD in M101A by NAPDH. (A) Time dependence of the reduction of the FAD in M101A by NAPDH. The reaction of oxidized M101A with NAPDH (15-500 μM) was monitored at 458 nm. The decrease in absorbance corresponds to flavin reduction. The solid lines are fits to Eq. 1. (B) Rate constants as a function of NADPH concentration.

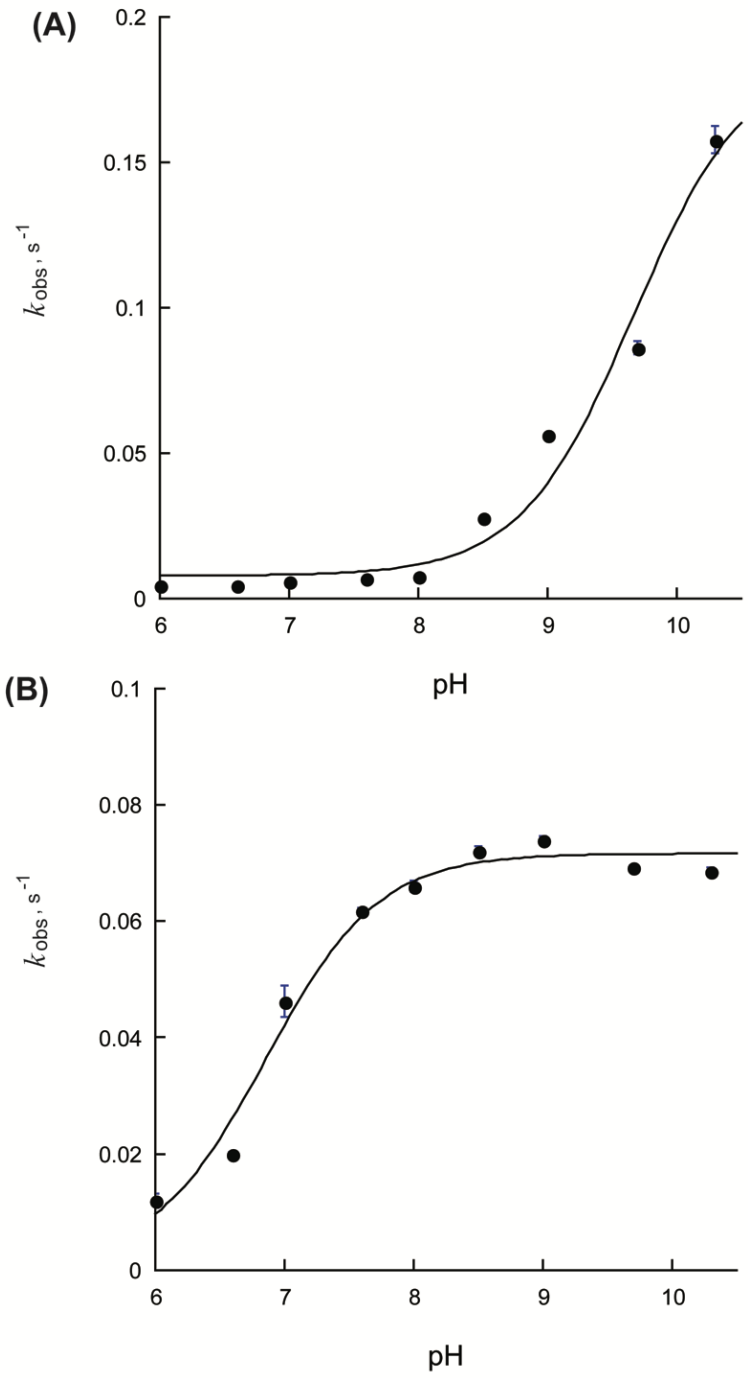

Figure S4. pH dependence of flavin oxidation in M101A monitored at 458 nm. Rate constant for flavin oxidation measured in the absence (A) or presence (B) of L-Orn. The data were fit to Eq. 3.

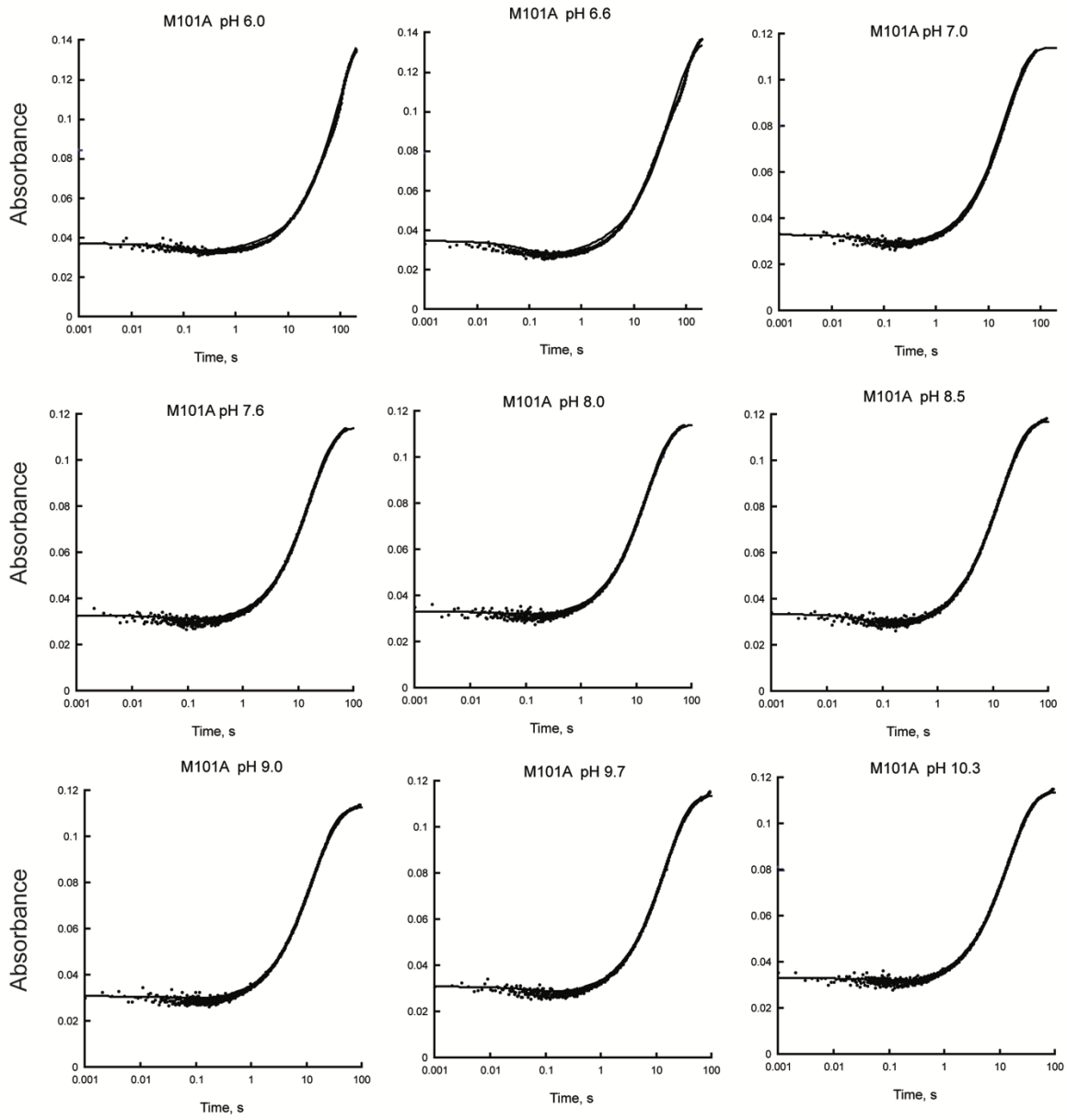

Figure S5. Oxidation of M101A monitored at 458 nm in the presence of L-Orn at various pH values. The data were fit to the triple exponential function in Eq. 2.

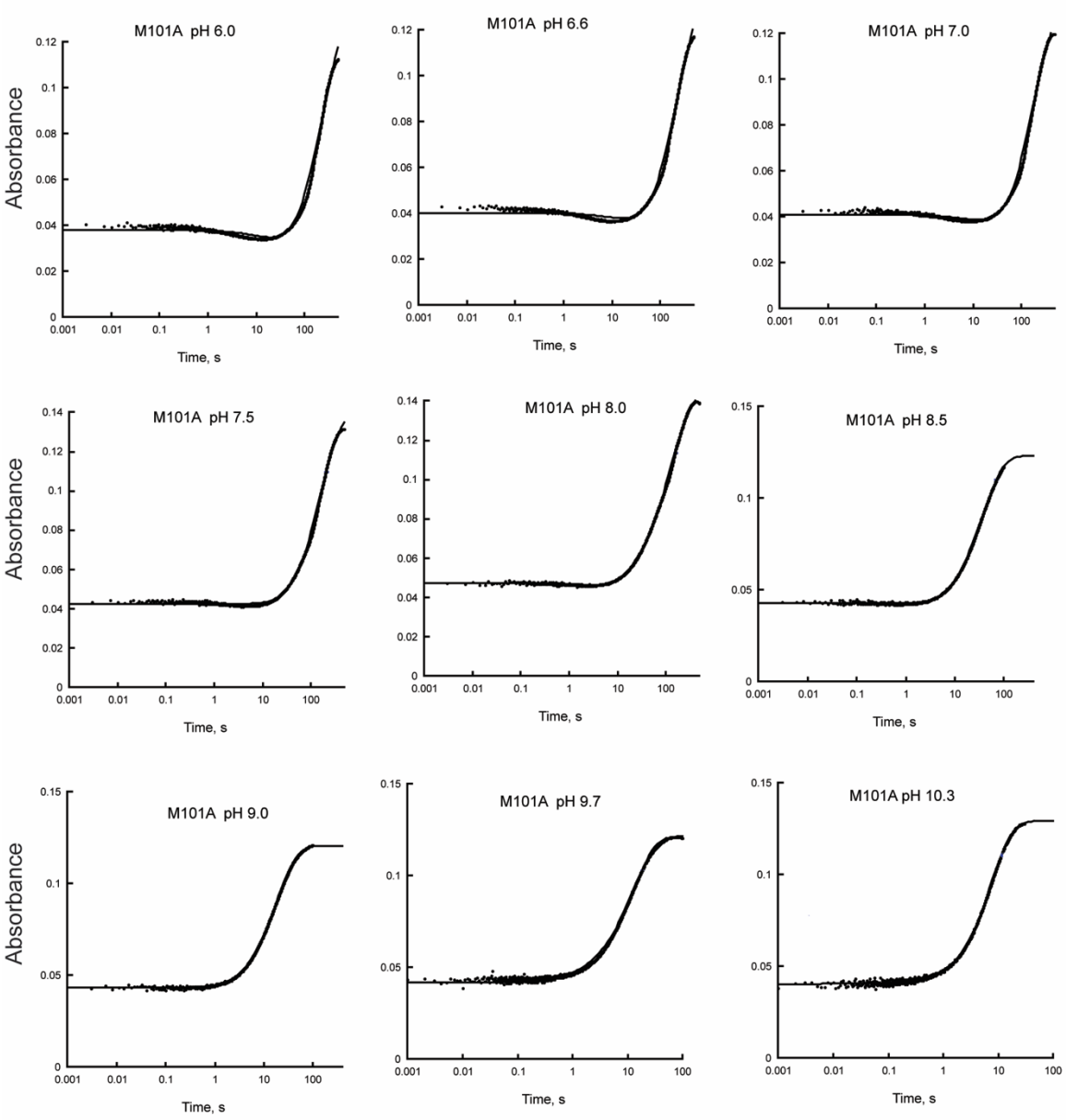

Figure S6. Oxidation of M101A monitored at 458 nm in the absence of L- Orn at various pH values. The data were fit to double exponential rise (Eq. 2 with the third term omitted).

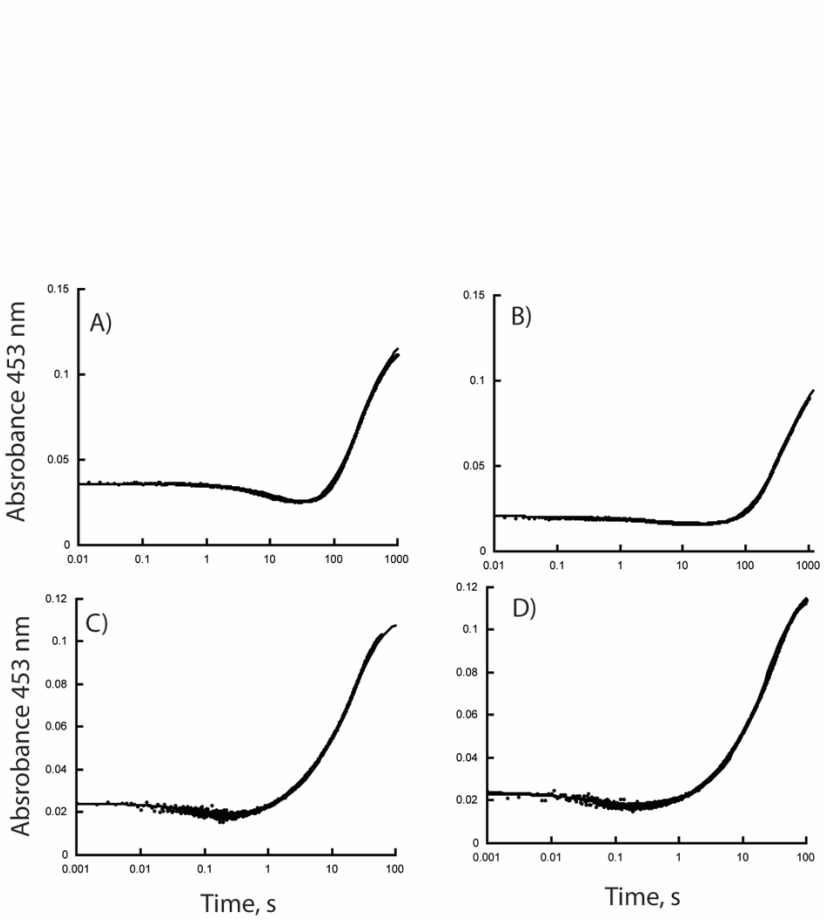

Figure S7. Oxidation of M101A at pD 7.0 in H_2O (A), pD 7.0 in D_2O (B), pD 9.0 in H_2O (C), or pD 9.0 in D_2O (D) (Experiment done in triplicate, only one sample is shown). The data were fit to the triple exponential function in Eq. 2.