Supporting Information for

"Electron transfer reactions of candidate tumor suppressor 101F6 protein, a cytochrome b561 homologue, with ascorbate and monodehydroascorbate radical "

by Mariam C. Recuenco, Md. Motiur Rahman, Fusako Takeuchi, Kazuo Kobayashi, and Motonari Tsubaki

FIGURE LEGENDS FOR SUPPLEMENTAL DATA

Figure S1. Time-dependent absorption changes on the reduction of recombinant human 101F6 with AsA monitored by a stopped-flow method at different pH and AsA concentrations. Purified recombinant human 101F6-H_8 protein (2 μM) in 50 mM potassium phosphate buffer (pH 5.0, 6.0, 7.0) containing 1 % β-octyl glucoside in one chamber of the stopped-flow apparatus and AsA (2, 4, 8, or 16 mM) dissolved in the same buffer in the other chamber were mixed in a mixing holder. Time-dependent absorption changes were monitored at 427 nm at 25°C. Final concentrations of the 101F6-H_8 protein and AsA were 1 μM and 1, 2, 4 or 8 mM, respectively (panel (A), (B), (C), and (D), respectively). The absorbance changes at 427 nm were plotted against time in a logarithmic scale.

Figure S2. Effect of DEPC-treatment of recombinant human 101F6 on the time-dependent absorption change for the heme reduction with AsA monitored by a stopped-flow method. Time-dependent absorption changes for the heme reduction with AsA of recombinant human 101F6-H_8 protein pre-treated with DEPC was measured in 50 mM potassium phosphate buffer (pH 7.0) containing 1 % β-octyl glucoside by a stopped-flow technique for 10 (panel A) and 60 sec (panel B) time domains. The absorbance changes at 427 nm were plotted against time in a logarithmic scale and were compared with those of control sample. The extent of the *N*-carbethoxylation upon the DEPC-treatment of 101F6-H_8 protein was estimated as 8.8 His residues/ molecule. Other conditions were described in the main text.

Figure S3. Absorbance changes of recombinant human 101F6-H_8 protein during the pulse radiolysis experiments. Recombinant human 101F6-H_8 protein (15 or 30 μM) in oxidized state in 50 mM potassium phosphate buffer (pH 7.0) containing 1 % (w/v) β-octyl glucoside was mixed with 10 mM (final) of AsA. The mixture in a quartz cell was first bubbled with N_2O gas, and the container was sealed. Then, the sample was irradiated with a high energy radiation pulse to produce the MDA radical in the solution. Just after the pulse, the changes in absorbance at wavelengths near the Soret band were recorded. The concentration of MDA radical was estimated based on the absorbance at 360 nm using the extinction coefficient of 3300 $M^{-1}cm^{-1}$ for the independent experiments without the protein. The experiments were performed at room temperature.

SUPLEMENTAL TABLE

Table S1. Apparent rate constants for the heme reduction of recombinant human 101F6-H_8 with AsA as reductant measured by a stopped-flow method.

All experiments were performed using 2 μM of 101F6-H_8 concentration at 25 °C. Results reported are means of four replicates. Values in parentheses are the standard deviations (SD).

Final AsA conc.	pH 5.0		pH 6.0		pH 7.0	
	k_{app} (s^{-1}) 1 sec	k_{app} (s^{-1}) 10 sec	k_{app} (s^{-1}) 1 sec	k_{app} (s^{-1}) 10 sec	k_{app} (s^{-1}) 1 sec	k_{app} (s^{-1}) 10 sec
1 mM	5.53 (0.55)	0.43 (0.02)	4.31 (0.17)	0.87 (0.04)	6.67 (0.65)	0.74 (0.02)
2 mM	4.57 (0.27)	0.43 (0.03)	4.23 (0.16)	1.02 (0.09)	4.24 (0.50)	0.75 (0.02)
4 mM	4.73 (0.10)	0.51 (0.01)	3.92 (0.47)	1.13 (0.13)	4.20 (0.52)	0.90 (0.04)
8 mM	4.49 (0.19)	0.73 (0.02)	4.78 (0.17)	1.29 (0.11)	3.50 (0.16)	1.09 (0.02)

APPENDIX

Transient kinetic analysis of the reduction of 101F6 with AsA by a stopped-flow method

For the analysis of the transient kinetics on the reduction of 101F6 with AsA by a stopped-flow method, we represented the reaction with the following scheme (1)

$$A + B \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} C \underset{k_{-2}}{\overset{k_{+2}}{\rightleftarrows}} D \qquad \cdots\cdots\cdots(1)$$

where A is the oxidized form of 101F6 and B is AsA. An intermediate complex C, the 101F6(Fe^{3+})•AsA complex, is formed by a reversible bimolecular reaction, which is followed by a reversible electron transfer reaction leading to the formation of the final product D, the reduced form of 101F6 (or more specifically, 101F6(Fe^{2+})•MDA complex). This two-step reaction can be simplified as scheme (2),

$$A + B \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} D \qquad \cdots\cdots\cdots(2)$$

where A is the oxidized form of 101F6 and B is AsA. Then, the reduced form of 101F6, product D, is formed by a reversible bimolecular reaction. As the first approximation of scheme (2), it can be treated as a pseudo first order reaction, since the concentration of AsA is great excess of the concentration of 101F6, and the equilibrium between three components is attained in the time domain of a later reaction stage. In this case, k_{app}, the apparent rate constant can be obtained by a following equation (3),

$$k_{app} = k_{+1}([A] + [B]) + k_{-1} \qquad \cdots\cdots\cdots(3)$$

Since we have already assumed that the concentration of B, [B], is far excess of the concentration of A, [A], we can further simplify the equation to obtain equation (4)

$$k_{app} = k_{+1}[B] + k_{-1} \qquad \cdots\cdots\cdots(4)$$

Therefore, from the plot of k_{app} *vs.* the concentration of B, (*i.e.*, the concentration of AsA), we can obtain the second order rate constant k_{+1} as the slope of the linear relationship and the dissociation constant k_{-1} as an intercept of the plot.

However, if we considered the actual electron transfer reaction from AsA to the oxidized heme of 101F6 protein more precisely, the scheme (2) might be too simplified. At least, we should consider the situation as described in scheme (1). In the scheme (1), which is actually the combination of a bimolecular reaction and a following single molecular reaction, two mutually related equations (5, 6) containing two apparent rate constants k_{app1} and k_{app2} for two relaxation processes in the scheme (2) can be obtained,

$$k_{app1} + k_{app2} = k_{+1}([A] + [B]) + k_{-1} + k_{+2} + k_{-2} \qquad \cdots\cdots\cdots(5)$$

$$k_{app1}k_{app2} = k_{+1}(k_{+2} + k_{-2})([A] + [B]) + k_{-1}k_{-2} \qquad \cdots\cdots\cdots(6)$$

We can make it simplified under a condition in which the bimolecular reaction is slower enough than the following single molecular reaction in scheme (1), or more specifically, under the

condition of $k_{+1}([A]+[B])+k_{-1} << k_{+2}+k_{-2}$

$$k_{app1} = k_{+2} + k_{-2} \qquad \text{.....(7)}$$

$$k_{app2} = k_{+1}([A]+[B]) + k_{-1}k_{-2} \Big/ (k_{+2}+k_{-2}) \qquad \text{.....(8)}$$

As already assumed to obtain the equation (4), the concentration of B, [B], is far excess of the concentration of A, [A], we can further simplify the equation (8) to obtain equation (9).

$$k_{app2} = k_{+1}[B] + k_{-1}k_{-2} \Big/ (k_{+2}+k_{-2}) \qquad \text{.....(9)}$$

From the plot of the apparent rate constant k_{app1} *vs*. concentration of B, (*i.e.*, AsA), we can obtain the second order rate constant k_{+1} and the intercept that corresponds to $k_{-1}k_{-2}/(k_{+2}+k_{-2})$.

Comparison of equations (9) and (4) shows that, in the scheme (2), the non-zero intercept includes the rates of the dissociation the 101F6(Fe^{3+})•AsA complex (k_{-1}), which is biased by the factor of

$$k_{-2} \Big/ (k_{+2}+k_{-2}) \qquad \text{.....(10)}$$

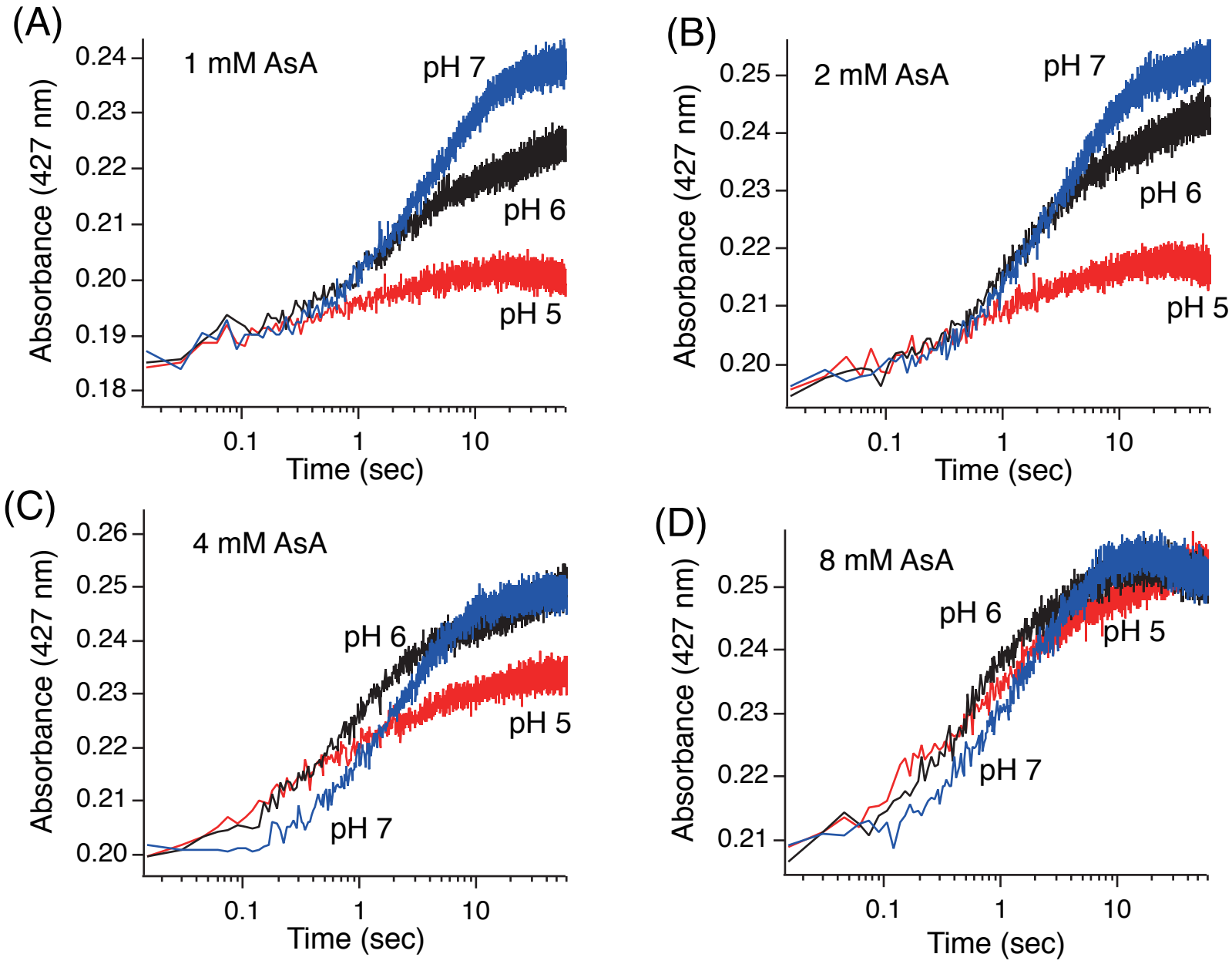

Figure S1 (Recuenco et al.)

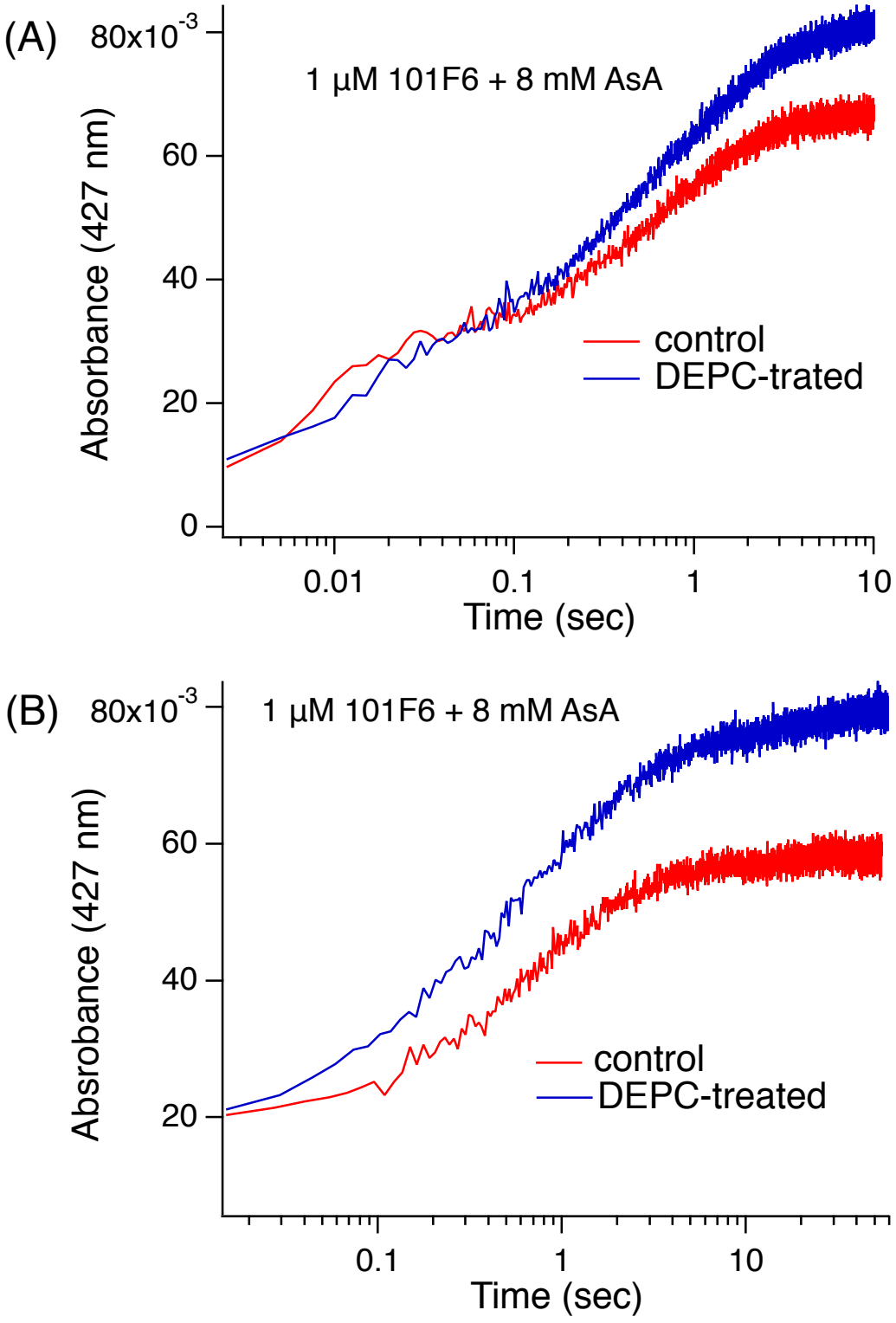

Figure S2 (Recuenco et al.)

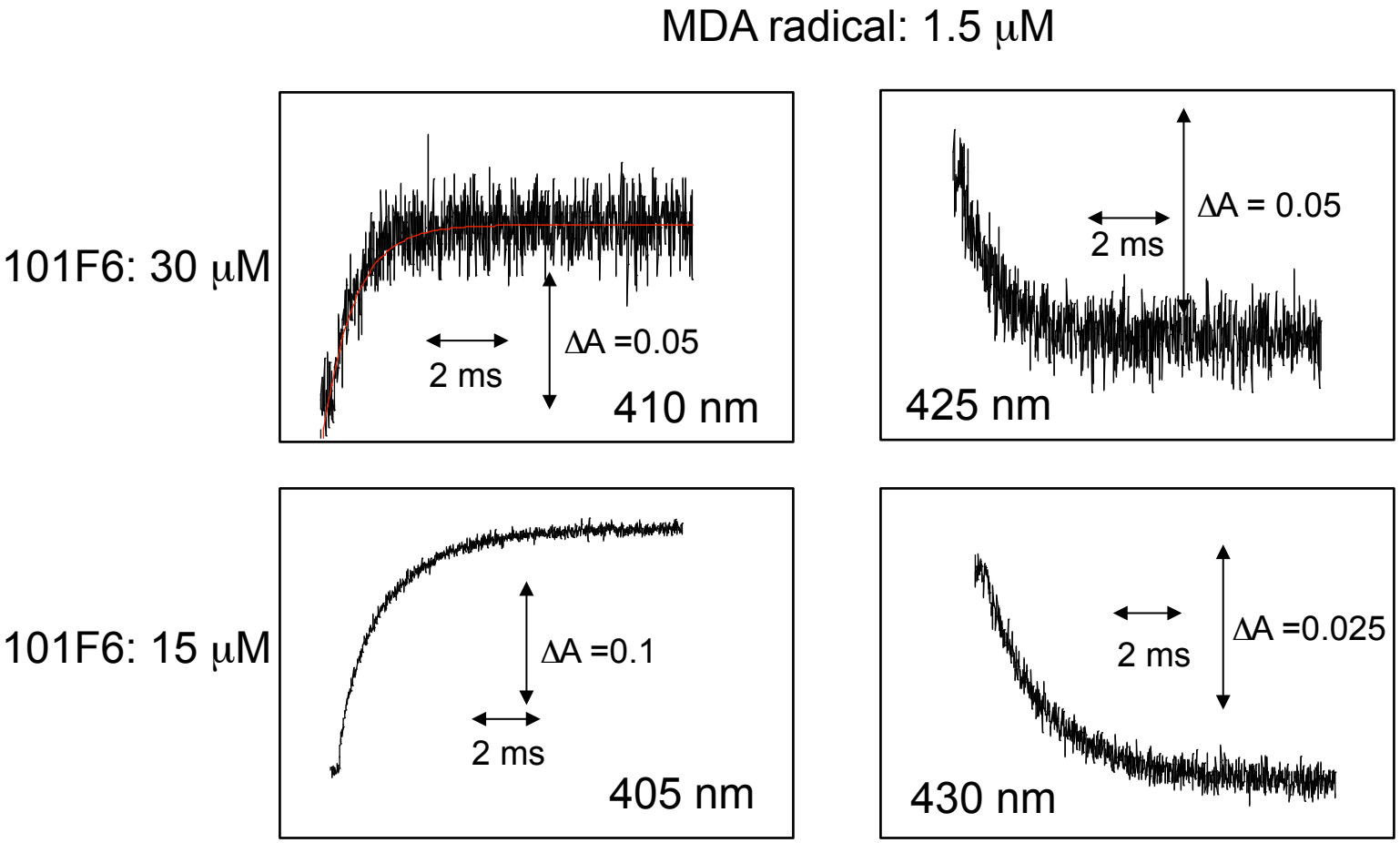

Figure S3 (Recuenco et al.)