Supporting Information for

Site-Specific Replacement of Y356 with 3,4-Dihydroxyphenylalanine in the β2 Subunit of *E. coli* Ribonucleotide Reductase

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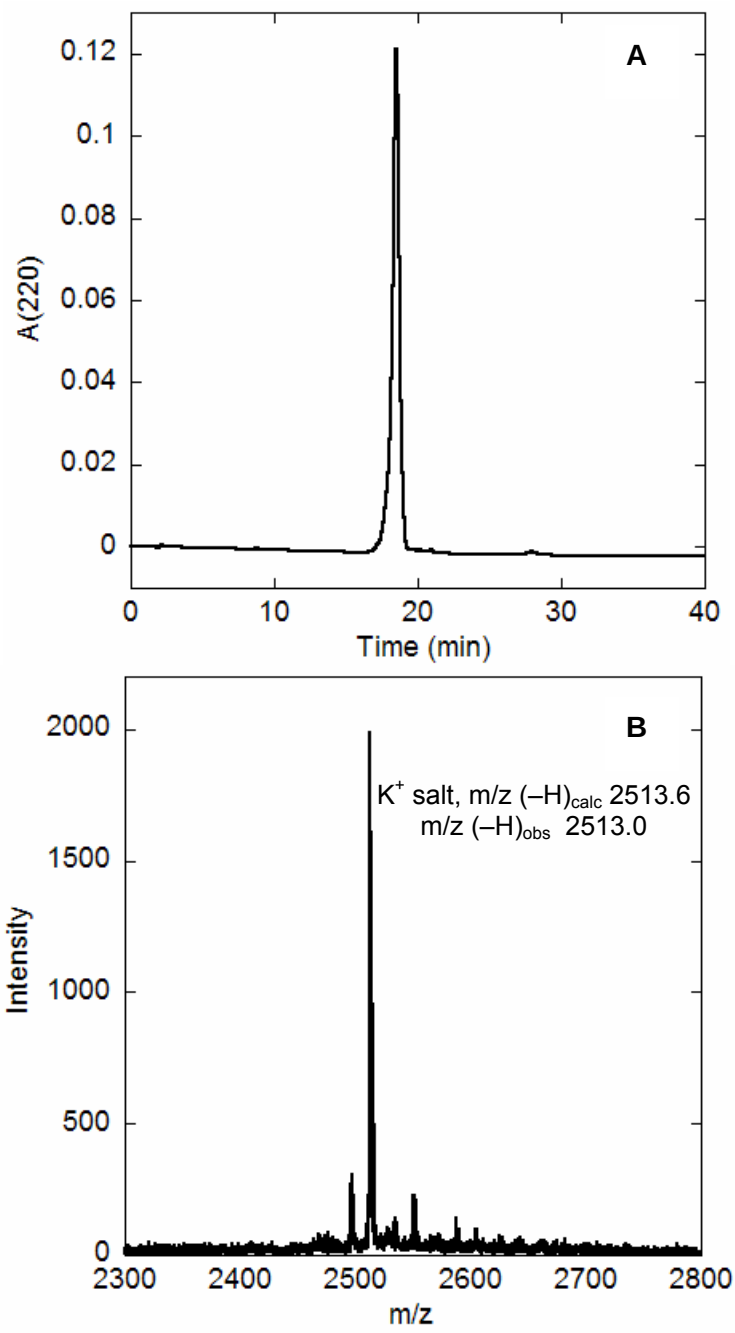

Figure S1. Characterization of DOPA amino acid-22mer used in the semi-synthesis of $DOPA_{356}$-β2. (A) Analytical HPLC profile using a Phenomenex Jupiter C18 column (4.6 mm x 150 mm) and a linear gradient of 10 % to 75 % acetonitrile versus 0.1 M ammonium bicarbonate, pH 6.8 (1 ml/min) over 45 min. Purification of the peptide and deprotection of the (t-Buthio)-protecting group were performed at pH 6.8 due to the base-sensitivity of DOPA. (B) MALDI-TOF MS of (t-Buthio)-deprotected DOPA-22mer just before ligation to the truncated protein. Minor peaks correspond to free acid form, Na^+ salt and combination of Na^+ and K^+ salts of the peptide.

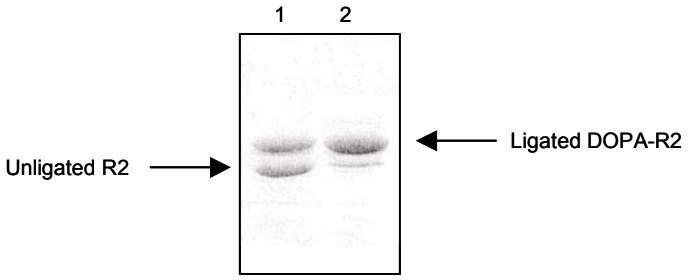

Figure S2. SDS gel of purified heterodimeric $DOPA_{356}$-β2 (Lane 1) and full-length homodmeric $DOPA_{356}$-β2 (Lane 2). Heterodimeric $DOPA_{356}$-β2 is a by-product of the semi-synthesis of full-length $DOPA_{356}$-β2 homodimer. The heterodimer consists of a truncated monomer and a full-length DOPA-containing monomer. See ref 6 in paper. Fmoc-DOPA(acetonide)-OH was purchased from Novabiochem. Peptide synthesis was carried out as in Ref. 6. The ligation reaction and purification of full-length homodimeric $DOPA_{356}$-β2 were carried out at pH 7.1 to avoid side reactions due to instability of DOPA at high pH. Note that β2 made by the intein procedure contains the mutations V353C/S354C in addition to the residue inserted at position 356.

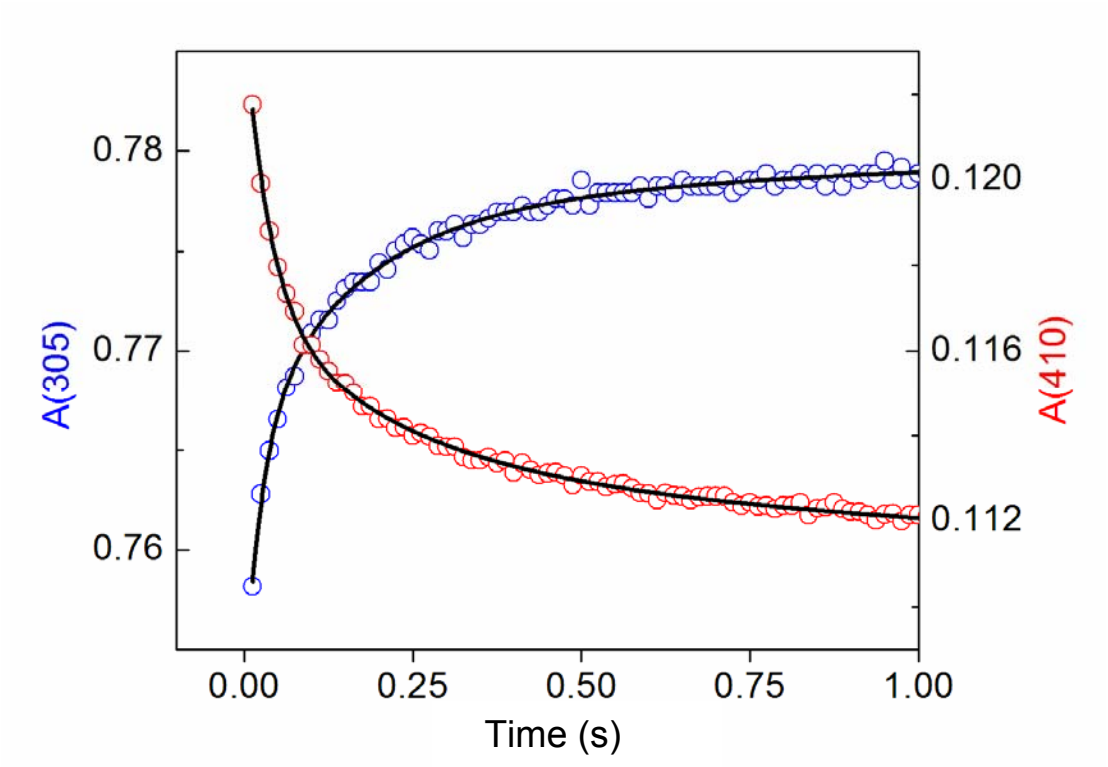

Figure S3. SF UV/Vis time course after $DOPA_{356}$-β2 (58 μM) and CDP (2 mM) in one syringe are mixed in a 1:1 ratio with pre-reduced α2 (58 μM) and ATP (6 mM) from another syringe. Fits to data (black lines) yield the kinetic parameters listed in Table 1. SF experiments were carried out on an Applied Photophysics DX. 17MV instrument using PMT detection at 305 nm for DOPA• formation and at 410 nm for Y• disappearance**.** All traces shown are the average of 6-10 shots.

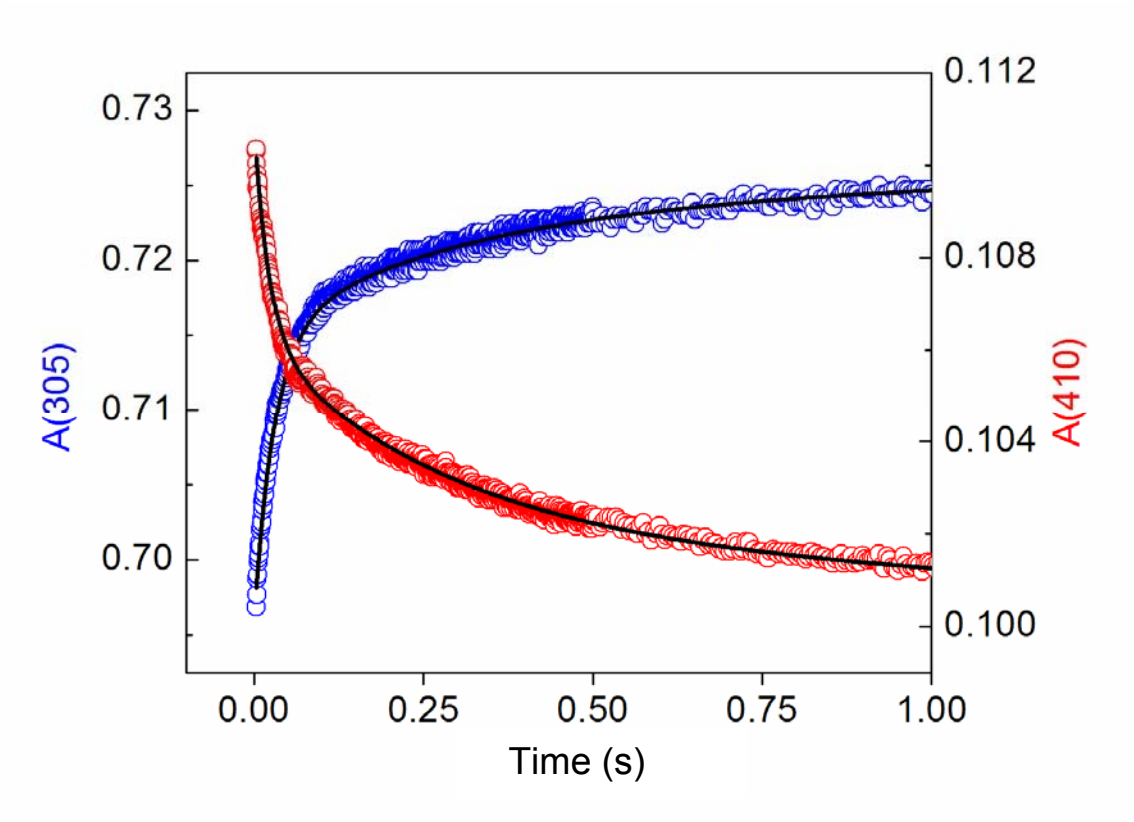

Figure S4. SF UV/Vis time course after $DOPA_{356}$-β2 (45 μM) and CDP (2 mM) in one syringe are mixed in a 1:1 ratio with pre-reduced α2 (45 μM) and TTP (200 μM) from another syringe. Fits to data (black lines) yield the kinetic parameters listed in Table 1.

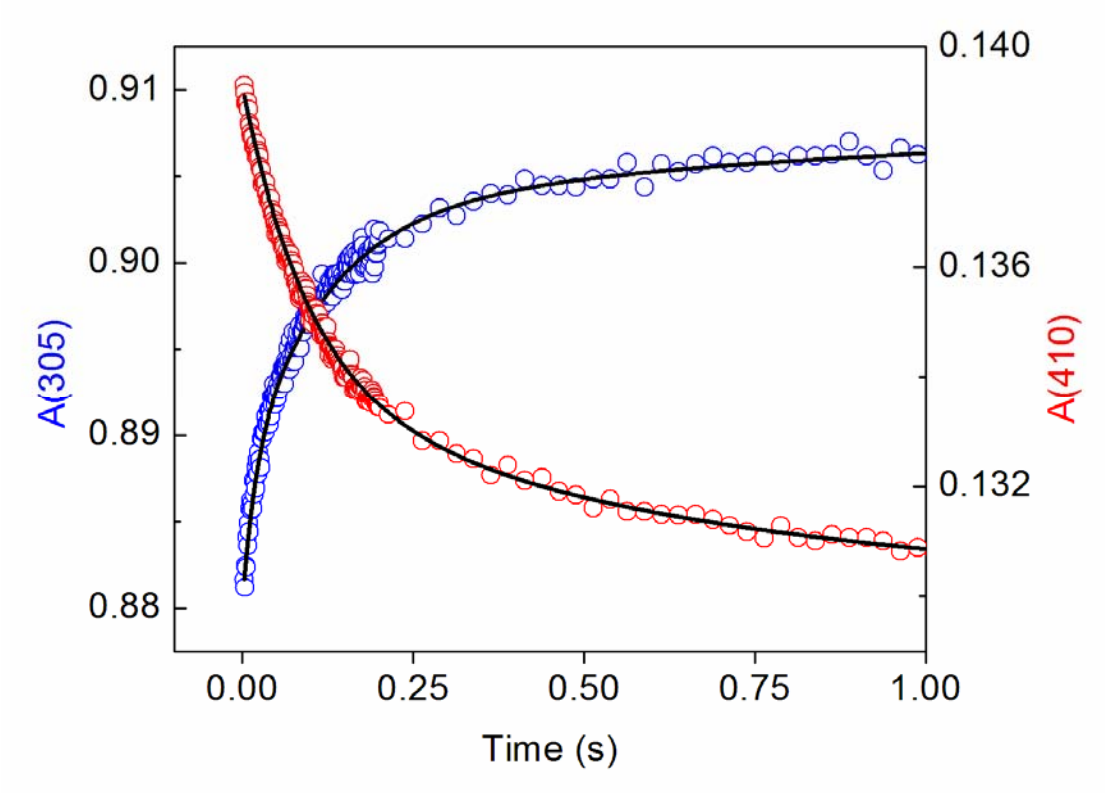

Figure S5. SF UV/Vis time course after $DOPA_{356}$-β2 (58 μM) and CDP (2 mM) in one syringe are mixed with pre-reduced α2 (58 μM) from another syringe. Fits to data (black lines) yield the kinetic parameters listed in Table 1.

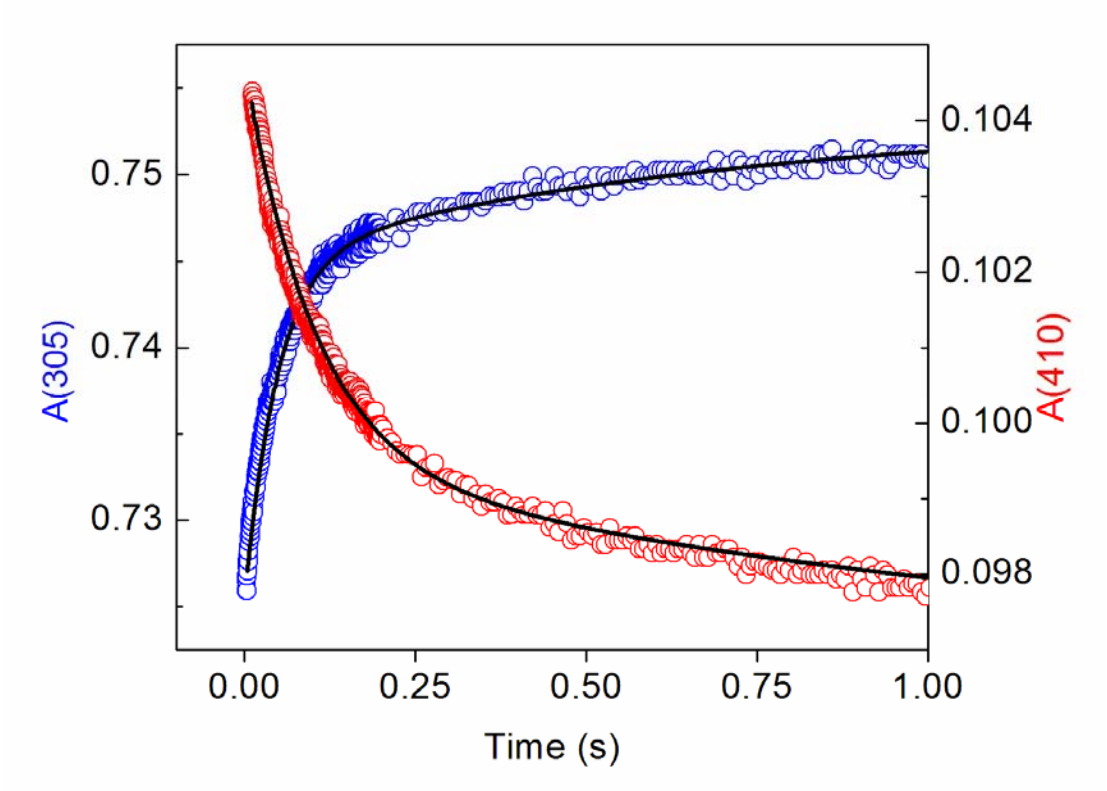

Figure S6. SF UV/Vis time course after $DOPA_{356}$-β2 (45 μM) and GDP (2 mM) in one syringe are mixed in a 1:1 ratio with pre-reduced α2 (45 μM) from another syringe. Fits to data (black lines) yield the kinetic parameters listed in Table 1.

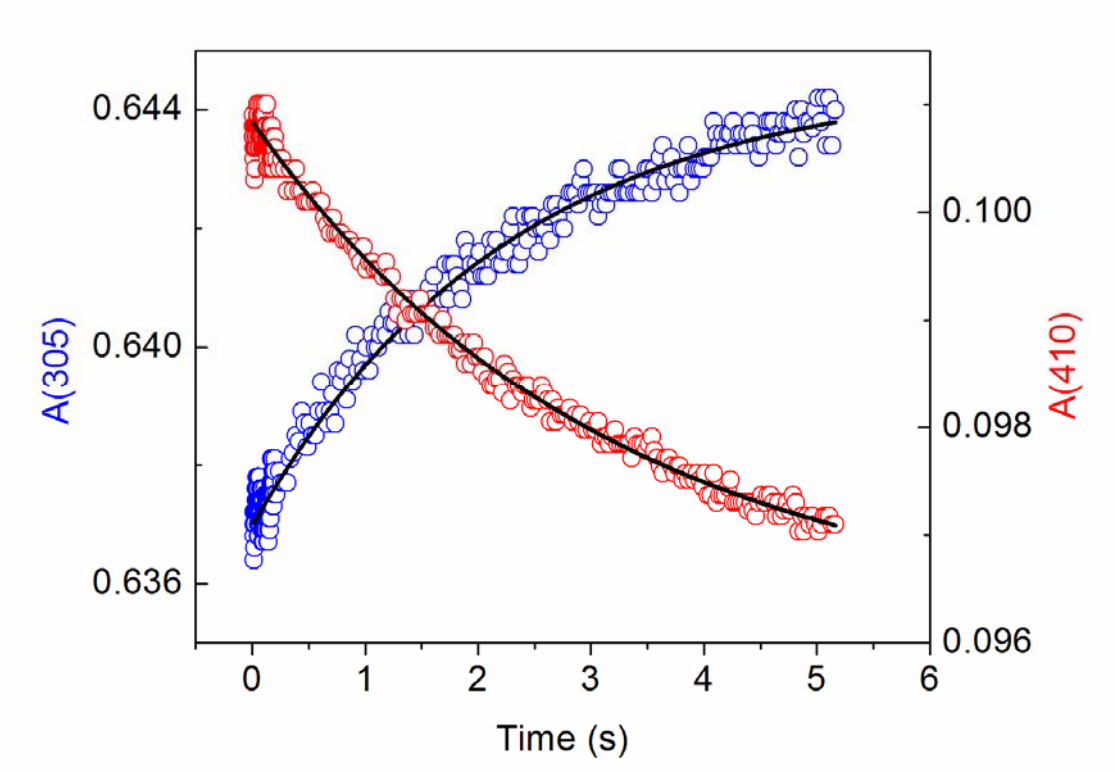

Figure S7. SF UV/Vis time course after $DOPA_{356}$-β2 (50 μM) in one syringe is mixed with α2 (50 μM) and TTP (200 μM) from another syringe. Fits to data (black lines) yield the kinetic parameters listed in Table 1.