Supporting Information

The formal reduction potential of 3,5-difluorotyrosine in a structured protein: Insight into multistep radical transfer

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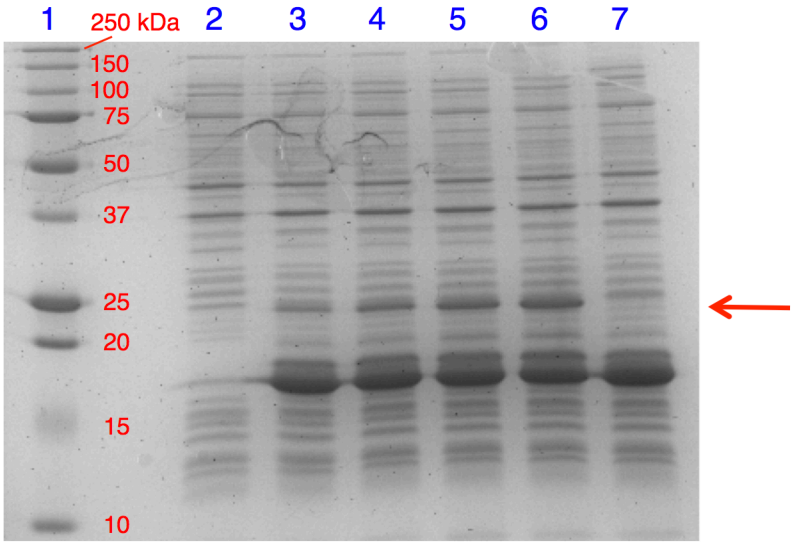

Figure S1. *SDS-PAGE analysis of $\alpha_3(3,5)F_2Y$ expression in E. coli BL21(DE3).* The SDS-PAGE (15%) gel displays in lane (1) molecular weight markers, lane (2) pre-induction sample, lane (7) post-induction sample of a culture to which no 3,5-F_2Y addition was made, and in lane (3-6) expression of $\alpha_3(3,5)F_2Y$ in cultures containing 0.5, 1.0, 1.5 and 2.0 mM 3,5-F_2Y respectively. The α_3X protein is expressed as a thioredoxin fusion. The calculated molecular weights of the truncated thioredoxin-α_3(residue 1-31) and full-length thioredoxin-$\alpha_3(3,5)F_2Y$ fusions are 17370 Da and 21348 Da, respectively. The band consistent with full-length thioredoxin $\alpha_3(3,5)F_2Y$ fusion protein is indicated with an arrow.

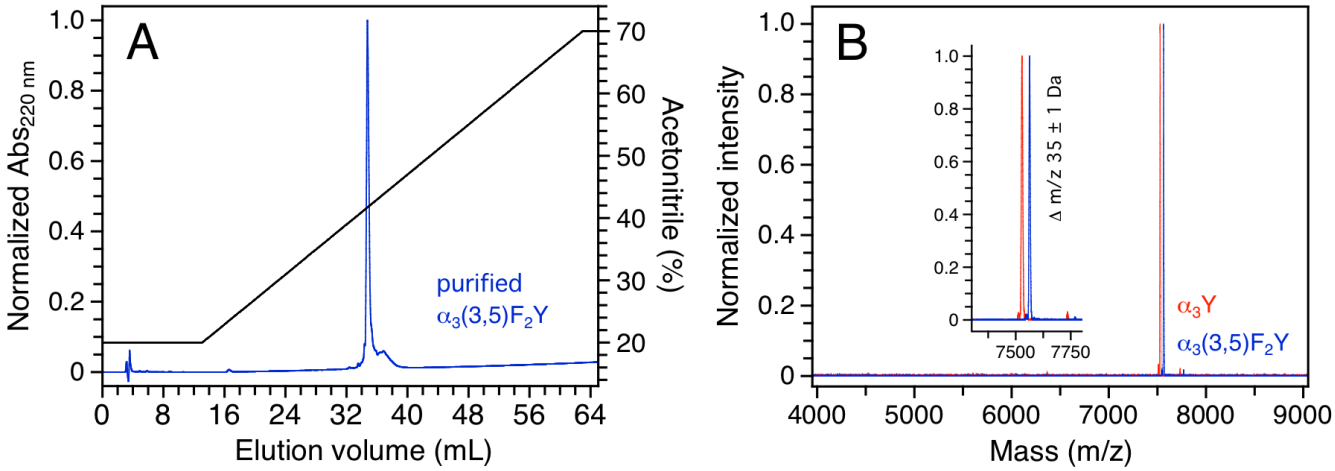

Figure S2. *Analytical HPLC and mass spectrometry evaluation of purified $\alpha_3(3,5)F_2Y$.* Panel (A) displays a typical analytical C18 reversed-phase chromatogram of purified $\alpha_3(3,5)F_2Y$. The freeze-dried protein was dissolved in 20 mM sodium acetate, pH 5.8, and eluted with a linear 20-70% acetonitrile gradient over 50 min. Panel (B) shows MALDI-TOF traces of purified $\alpha_3(3,5)F_2Y$ (blue) and α_3Y (red). Each trace displays a single major peak, whose maxima are separated by 35 ± 1 Da (insert). This is consistent with the exchange of Y_{32} to 3,5-F_2Y_{32} (calculated $\Delta m/z$ = 36 Da).

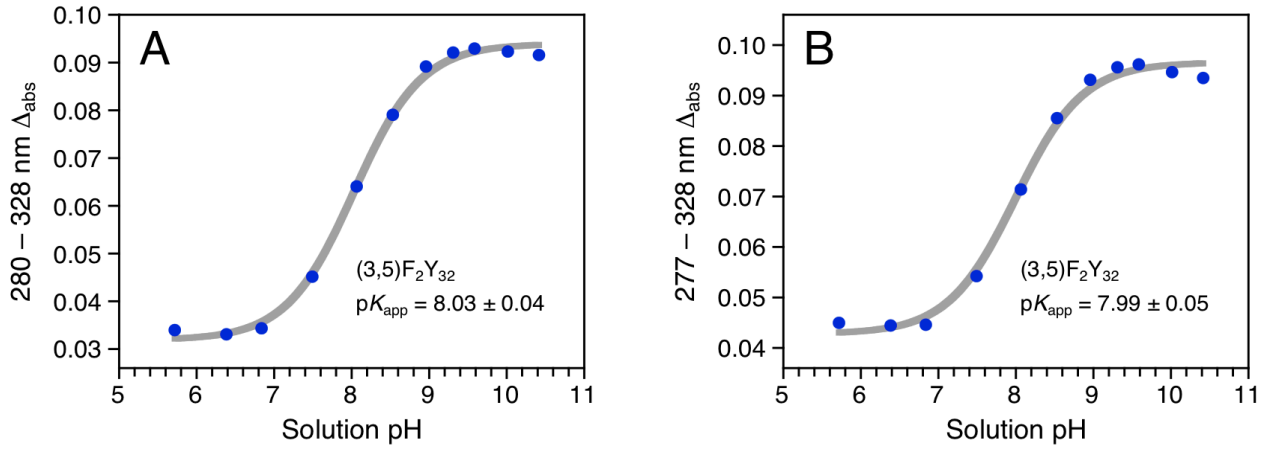

Figure S3. *Fitting the UV-Vis pH-titration curve of $\alpha_3(3,5)F_2Y_{32}$.* The pK_{app} of (3,5)F_2Y_{32} was estimated by fitting the raw absorption (measured at 280 and 277 nm) and baseline-subtracted (baseline points measured at 328 and 400 nm) absorption of (3,5)F_2Y_{32}-O^- as a function of pH to a single pK_a. The non-linear curve fitting routines in KaleidaGraph (www.synergy.com) were used for the data fitting. Panel (A) (delta 280 – 328 nm absorption) and panel (B) (delta 277 – 328 nm absorption) display two examples from the fitting analysis. No significant difference was found in the estimated pK_{app} value from the 280 or 277 nm ± baseline fits (average pK_{app} of 7.98 with an average fitting standard error of 0.06).

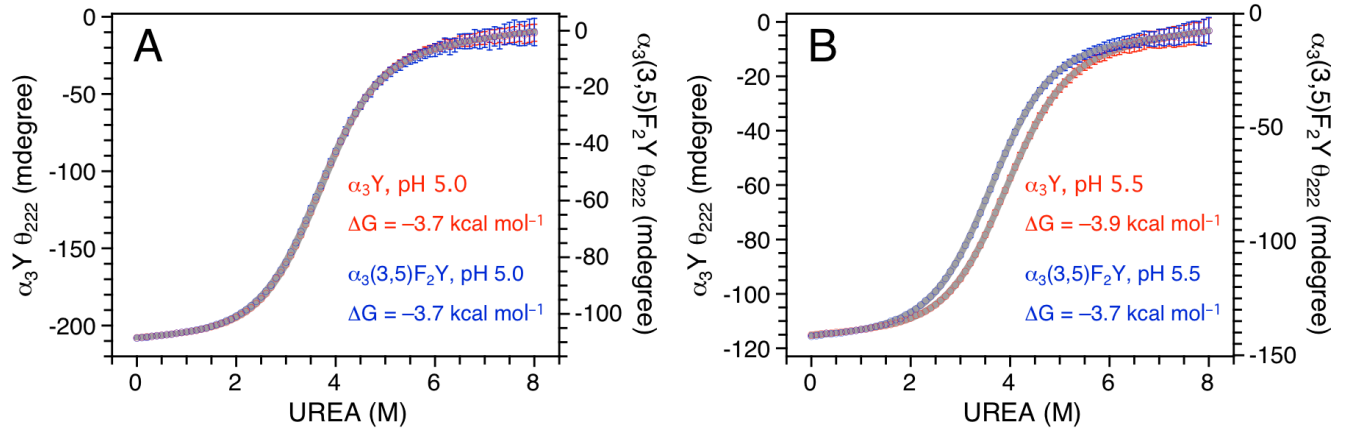

Figure S4. *Chemical denaturation of α_3Y and $\alpha_3(3,5)F_2Y$.* The figure displays urea-induced unfolding/folding transitions of $\alpha_3(3,5)F_2Y$ (blue) and α_3Y (red) obtained at (A) pH 5.0 and (B) pH 5.5, respectively. The grey lines represent nonlinear curve fits to determine the stability of the protein in the absence of denaturant.[1] Fitting standard error < ± 0.03 kcal mol^{-1}.

(1) Santoro , M. M., and Bolen, D. W. (1988) Unfolding free energy changes determined by the linear extrapolation method. 1. Unfolding of phenylmethanesulfonyl α-chymotrypsin using different denaturants. Biochemistry 27, 8063–8068.).

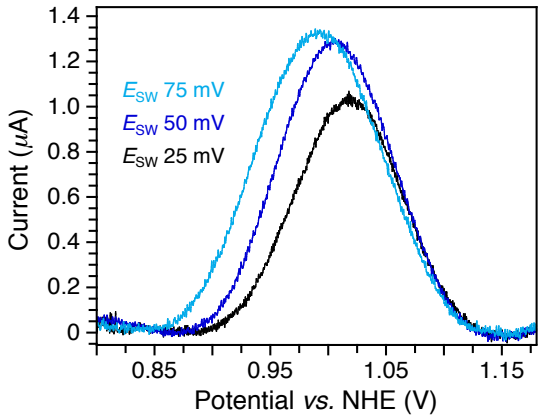

Figure S5. I_{net} of $\alpha_3(3,5)F_2Y$ as a function of the square-wave pulse amplitude. The traces were recorded using a SW frequency of 120 Hz and a pulse amplitude (E_{SW}) of 25 (black), 50 (blue) and 75 (light blue) mV. The insensitivity in the I_{net} lineshape to the pulse amplitude is consistent with diffusion-controlled electrode kinetics.[2,3] SWV settings: 90 μM $\alpha_3(3,5)F_2Y$ in 20 mM sodium acetate, 20 mM potassium phosphate, 20 mM sodium borate, 75 mM KCl, pH 5.70; PGE working electrode, temperature 25° C, step potential 0.15 mV, and SW frequency 120 Hz.

(2) Jeuken, L. J. C., McEvoy, J. P., and Armstrong, F. A. (2002) Insights into Gated Electron-Transfer Kinetics at the Electrode-Protein Interface: A Square Wave Voltammetry Study of the Blue Copper Protein Azurin. *J. Phys. Chem. B 106,* 2304–2313.

(3) Mirčeski, V., Komorsky-Lovrić, Š., and Lovrić, M. (2007) Square-wave voltammetry: Theory and applications. In Scholz F (ed) Monographs in electrochemistry (Springer-Verlag, Berlin, Germany).