Supplemental Information

The Solution Structure of the Regulatory Domain of Tyrosine Hydroxylase
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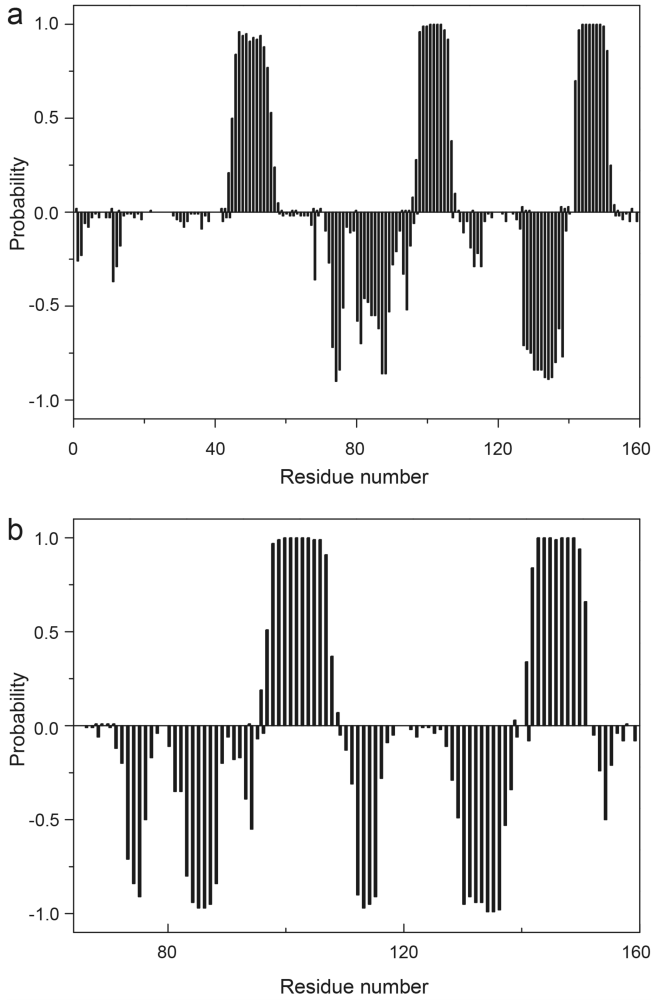

Figure S1. Secondary structure prediction for RDTyrH (a) and RDTyrH$_{65\text{-}159}$ (b) using PECAN.[1] The probabilities of α-helices and β-strands are given as positive and negative values, respectively.

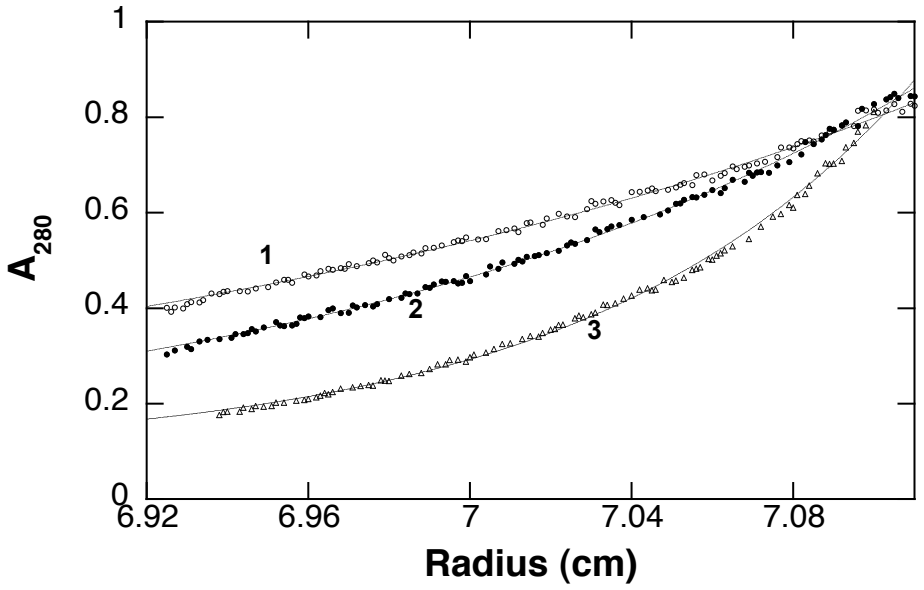

Figure S2. Equilibrium sedimentation ultracentrifugation of RDTyrH; conditions: 11,500 rpm (1), 14,000 rpm (2), or 20,000 rpm (3) in 0.24% octyl glucoside, 15 mM potassium phosphate, 38 mM ammonium sulfate, pH 6.5. The lines are from fits of the data to $A_r = A_0 \left(\frac{(1-\bar{v}\rho)\omega^2}{2RT} M (r^2 - r_0^2) \right)$, which describes the behavior of a single species with a value for M of 38,000 ± 1,000; A_r is the absorbance at distance r, A_0 is the absorbance at reference distance r_0, ω is the angular velocity in radians/s, ν is the partial specific volume calculated from the amino acid content, and ρ is the buffer density. There was no improvement in the quality of the fit when equations describing equilibria between monomers and larger species were used.

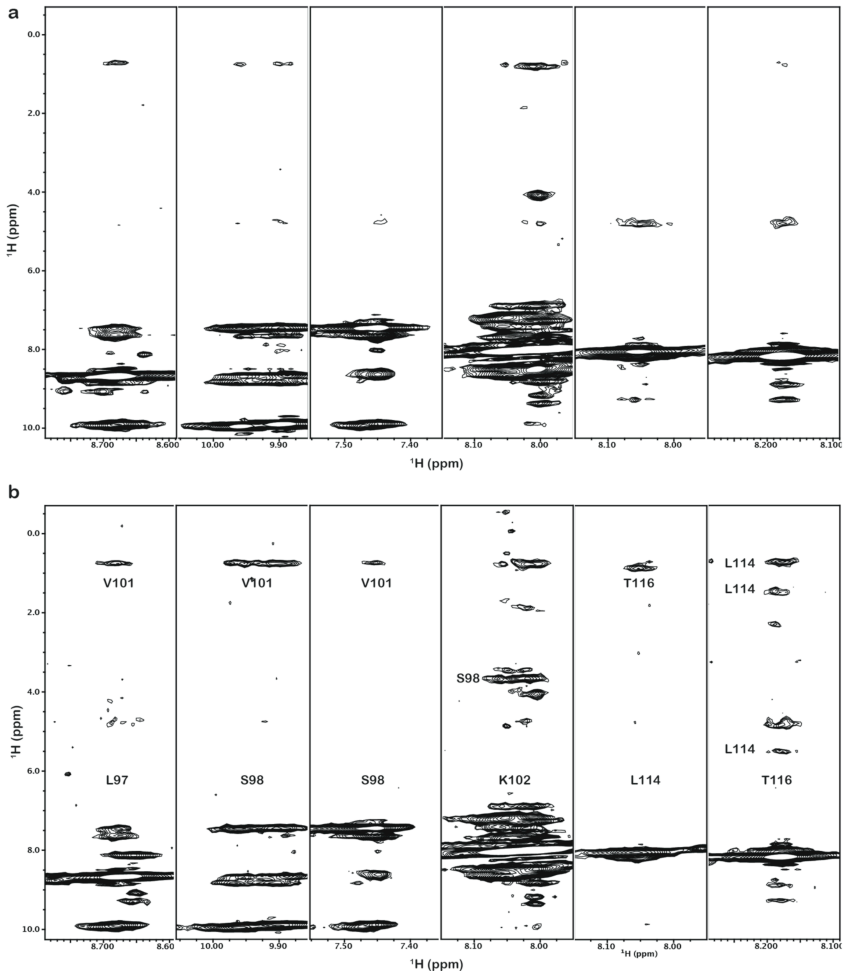

Figure S3. Selected ^{15}N slices of 3D NOESY-HSQC spectra of RDTyrH$_{65\text{-}159}$. (a) The spectra of the completely deuterated ^{15}N labeled RDTyrH$_{65\text{-}159}$, which show the crosspeaks between amide protons to H_2O. (b) The spectra of the heterodimer of completely deuterated ^{15}N labeled and unlabeled monomers, which also contain the crosspeaks between amide protons and the aliphatic protons of the unlabeled monomer. Amide proton assignments are provided at the bottom of each 2D slice, and the crosspeak assignments are indicated within the spectrum.

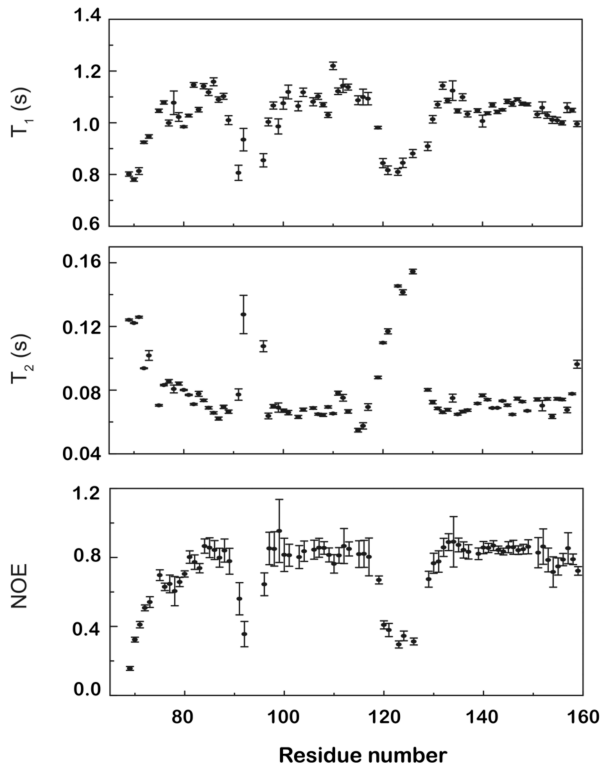

Figure S4. Backbone relaxation data for $RDTyrH_{65\text{-}159}$. The longitudinal relaxation time (T_1), transverse relaxation time (T_2), and heteronuclear ^{1}H-^{15}N NOE values versus the amino acid sequence of $RDTyrH_{65\text{-}159}$ were recorded at a magnetic field strength of 14.1 T (600 MHz ^{1}H) at 300 K.

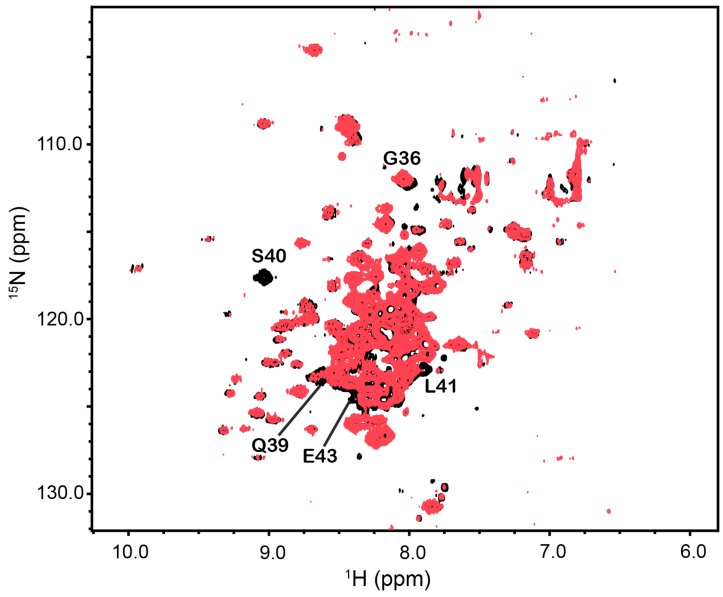

Figure S5. Overlay of the 2D ^{1}H-^{15}N HSQC spectra of RDTyrH (red) and RDTyrH phosphorylated at Ser40 (black). Residues with different chemical shifts in the two spectra are indicated.

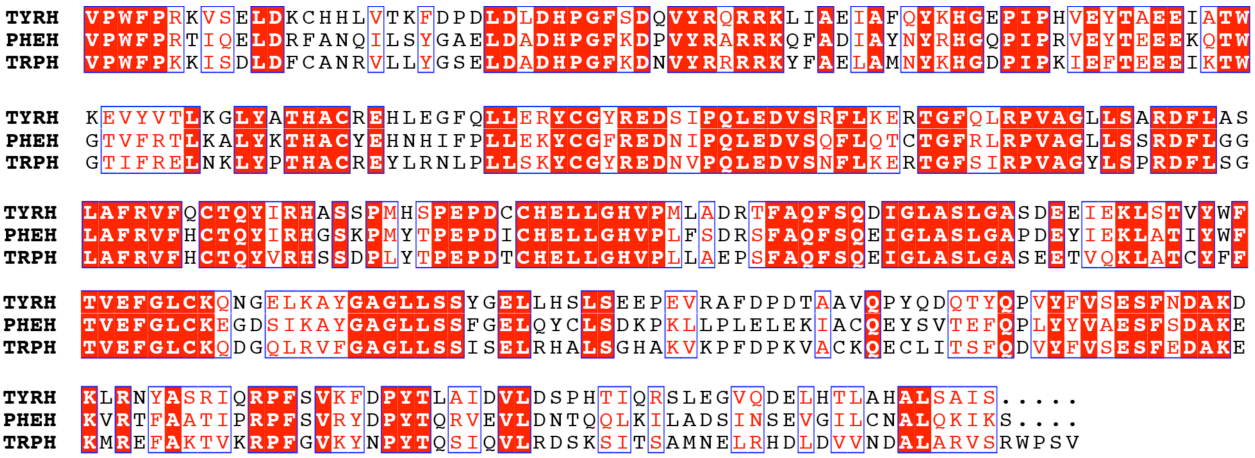

Figure S6. Sequence alignment of the catalytic domains of the three aromatic amino acid hydroxylases. The sequences begin with residue 161 of rat TyrH, residue 115 of rat PheH, and residue 102 of rat TrpH. The alignment was performed with the program Clustal X[2] and the figure was generated using ESPript.[3]

References

1. Eghbalnia, H. R., Wang, L., Bahrami, A., Assadi, A. & Markley, J. L. (2005). Protein energetic conformational analysis from NMR chemical shifts (PECAN) and its use in determining secondary structural elements. *J Biomol NMR* **32**, 71-81.
2. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876-82.
3. Gouet, P., Courcelle, E., Stuart, D. I. & Metoz, F. (1999). ESPript: analysis of multiple sequence alignments in PostScript. *Bioinformatics* **15**, 305-8.