

Supplementary Information (SI)

for

***Bacillus anthracis* Prolyl 4-Hydroxylase Interacts with and Modifies Elongation Factor Tu**

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Running title: Promiscuous substrate recognition by BaP4H

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Supporting Information Tables

Supporting Table 1. SAXS data and shape reconstruction statistics. The radius of gyration (R_g) and maximum dimension (D_{\max}) of BaP4H-BaEFTu complex and the individual proteins. The R_g calculated using two different methods (Guinier analysis and Autognom/pair distribution function (P_r)), are similar to each other.

| | BaP4H | BaEFTu | 1:1 complex |
|---------------------|----------|---------|-------------|
| R_g (Å) (Guinier) | 25 | 26 | 28 |
| $Q \cdot R_g$ | 0.49-1.3 | 0.4-1.3 | 0.3-1.3 |
| R_g (Å) (P_r) | 25 | 26 | 29 |
| D_{\max} (Å) | 78 | 91 | 110 |
| DAMMIF Symmetry | P2 | P1 | P1 |
| Mean Damaver NSD* | 0.64 | 0.57 | 0.57 |

* = Normalized Spatial Discrepancy: Diagnostic parameter for degree of similarity between sets of three-dimensional points.¹

Supporting Figures.

Figure S1. Conservation of residues between BaP4H and several other P4Hs. The darkest color (maroon) is the most conserved residues followed by the lighter magenta color progressing to the darkest cyan color (least conserved residues), are shown as a gradient. The highest conserved region is located around the active site. Conservation scores were generated from 50 unique P4H sequences and displayed on the BaP4H structure (PDB 5IAT) using ConSurf.²

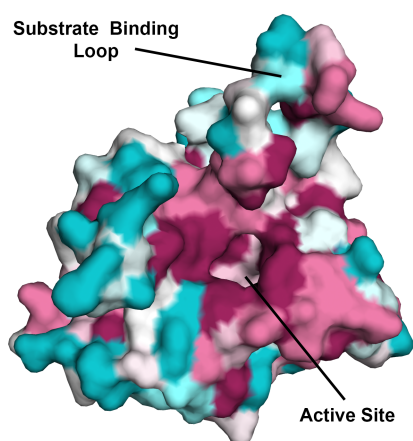


Figure S2. Gel filtration calibration curve using Superdex 200 10/300 GL column in the absence and presence of 200 mM NaCl.

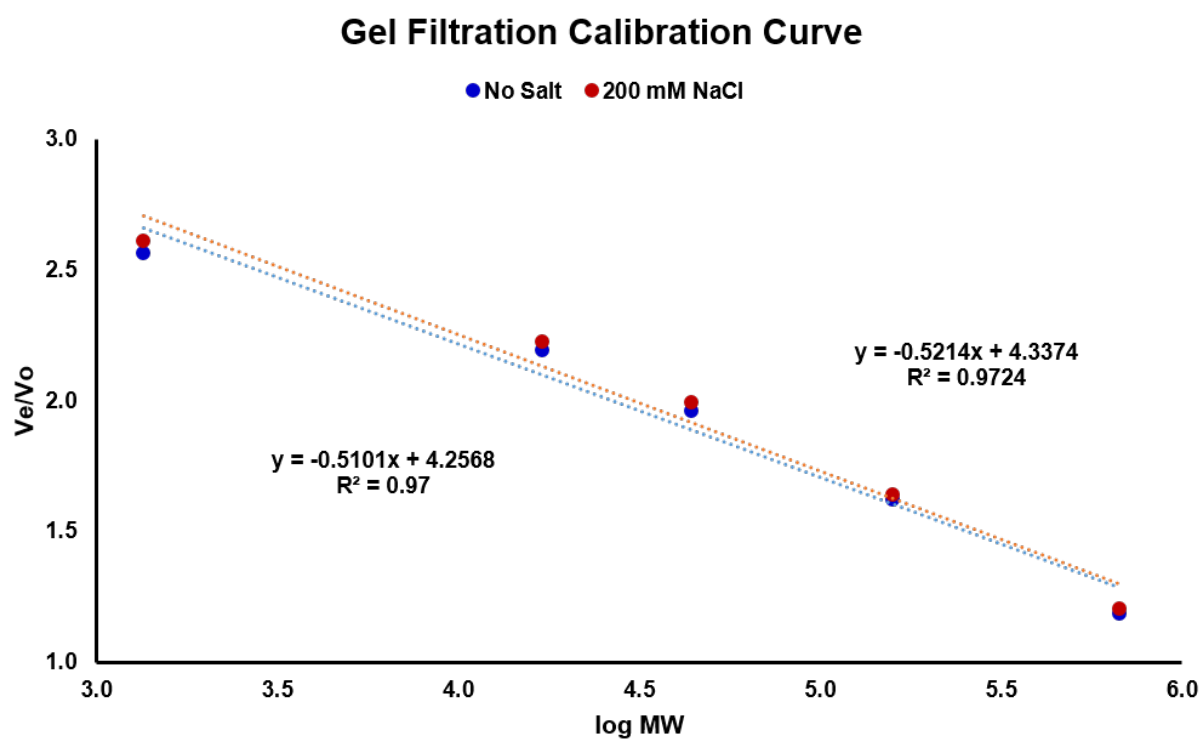


Figure S3. Chromatograms for size-exclusion chromatography-coupled small-angle X-ray scattering (SEC-SAXS) runs representing the three species – free BaP4H (blue), free BaEFTu (green) and 1:1 mixture of BaP4H-BaEFTu (black). For the complex, BaP4H and BaEFTu were added in the complex buffer (50 mM HEPES (pH 7.5), 10 mM MgCl₂, and 1 μ M GDP) to final concentrations of 80 μ M each. Free BaP4H or BaEFTu at 10 mg/mL each were loaded on to the column.

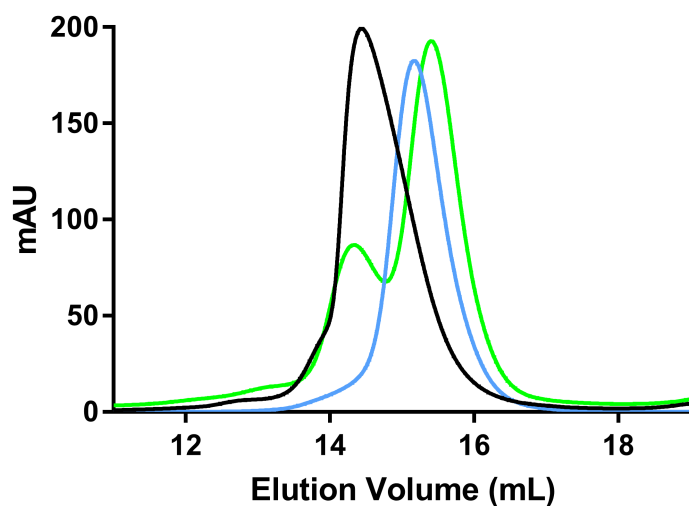
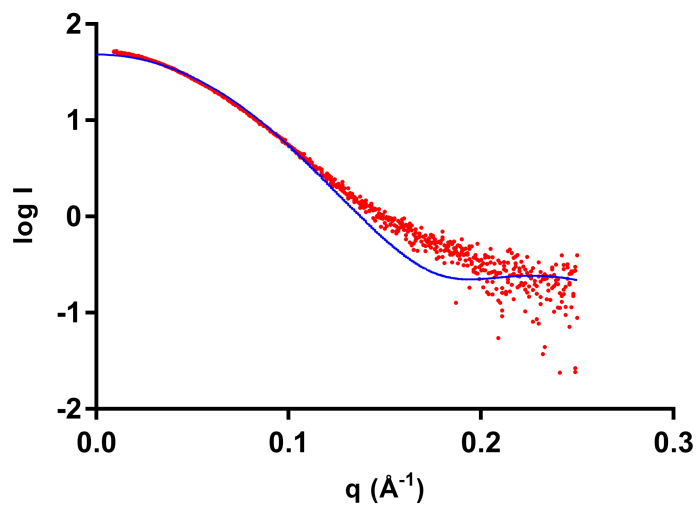


Figure S4. Theoretical scattering curve (blue) of BaP4H dimer calculated using CRY SOL, fit poorly (χ^2 of 18.15) with the observed scattering profile for the 1:1 complex of BaP4H-BaEFTu.



References

1. Kozin, M. B., and Svergun, D. I. (2001) Automated matching of high- and low-resolution structural models. *J. Appl. Cryst.* 34, 33-41.
2. Landau, M., Mayrose, I., Rosenberg, Y., Glaser, F., Martz, E., Pupko, T., Ben-Tal, N. (2005) ConSurf 2005: the projection of evolutionary conservation scores of residues on protein structures. *Nucleic Acids Res.* 33 (Web Server issue), W299-302.