Supporting Information

The N-B Interaction through a Water Bridge: Understanding the Chemoselectivity of a Fluorescent Protein Based Probe for Peroxynitrite

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Table S1. Oligonucleotides used in this study.

Primer name	Nucleotide Sequence
pnGFP-F	5'- TTTTTGGGCTAACAGGAGGAATTAACCATG -3'
pnGFP-R	5'- CAGCCAAGCTTAATGGTGATGGTGATGGTG -3'
pBAD-F	5'- ATGCCATAGCATTTTTATCC -3'
pBAD-R	5'- GATTTAATCTGTATCAGG -3'
pnGFP-T173G-F	5'- CTCGTGACCACCTTGGGCTAGGGCGTGCAGTGC -3'
pnGFP-T173G-R	5'- GCACTGCACGCCCTAGCCCAAGGTGGTCACGAG -3'
pnGFP-T64F-F	5'- CACTACCTGAGCTTCCAGTCCGTGCTG -3'
pnGFP-T64F-R	5'- CAGCACGGACTGGAAGCTCAGGTAGTG -3'
pnGFP-S66A-F	5'- CACTACCTGAGCACCCAGGCCGTGCTGAGCAAAGACCCC -3'
pnGFP-S66A-R	5'- GGGGTCTTTGCTCAGCACGGCCTGGGTGCTCAGGTAGTG -3'
pnGFP-H9T-F	5'- TTTTTGGGCTAACAGGAGGAATTAACCATGGGCTCGAGCACTTACAACAGCA CCAAGGTC -3'

Table S2. Crystallographic data, refinement and atomic model statistics.

Protein	pnGFP1.5-Y.Cro
Total reflections	1,606,287
Unique reflections observed	14,821
Space Group	P 43 21 2
Cell dimensions (a, b, c) (Å)	57.26, 57.26, 185.39
Resolution (Å)[a]	25.-2.75 (2.80-2.75)
Completeness[a] (%)	99.9 (100.0)
Average I/σ^{a}	80.4 (8.0)
R_{merge}[a,b]	0.089 (0.926)
R_{pim}, $CC_{1/2}$, CC* (shell)[c]	(0.129),(0.982),(0.995)
Atomic model statistics	
Asymmetric unit	1 protein chain, 16 H_2O
Crystallographic *R*-factor[d] (shell 3.17-2.75)	0.203 (0.249)
R-free	0.293 (0.336)
R-factor, all data combined	0.210
Average B-factors, all atoms ($Å^2$)	74.6
rms bond lengths (Å)	0.010
rms bond angles (degrees)	1.44

[a] Values in parentheses indicate statistics for the highest resolution shell.

[b] $R_{merge} = \Sigma_i \Sigma_j (I_{ij} - \langle I \rangle_i)/\Sigma_i \Sigma_j \langle I \rangle_i$, where I_{ij} is the amplitude of the jth observation of reflection i and $\langle I \rangle_i$ is the mean value of observations I_{ij}.

[c] For definitions of these quantities see Karplus & Diederichs[1]

[d] *R*-factor = $\Sigma||F_o|-|F_c||/\Sigma|F_o|$, where F_o and F_c are the observed and calculated structure amplitudes.

[1]. Karplus, P. A.; Diederichs, K., Linking Crystallographic Model and Data Quality. *Science* **2012,** 336, 1030-1033.

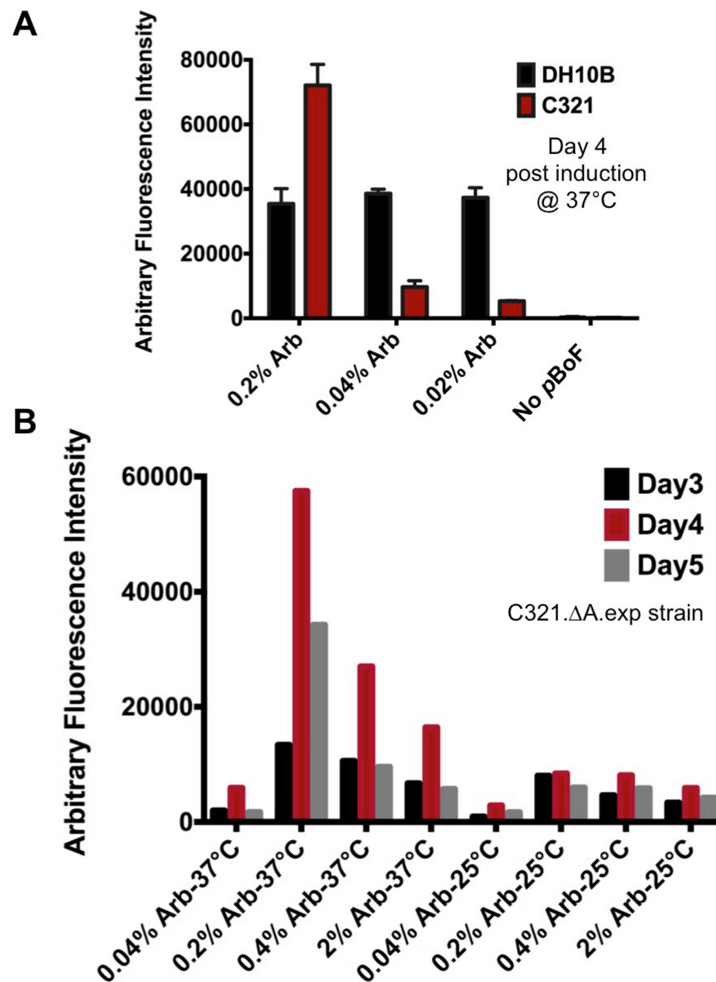

Figure S1. Optimization of conditions for preparation of *p*BoF-containing proteins. To optimize the expression condition, the plasmid pBAD-mCherry$_{151TAG}$ was used to co-transform DH10B or C321.ΔA.exp *E. coli* cells along with the plasmid pEvol-*p*BoF. Cells were grown on LB agar plates containing 100 μg/mL ampicillin and 50 μg/ml chloramphenicol at 37°C overnight. Individual colonies from the plates were then inoculated in 2 mL TB containing 1 mM *p*BoF, 100 μg/mL ampicillin, 50 μg/ml chloramphenicol and various concentrations of L-arabinose (0.02–2%). Cells were next grown at 25°C or 37°C, and fluorescence intensities of corresponding cell lysates were quantified each day thereafter. To prepare cell lysate, 500 μL of cell culture was pelleted by centrifugation and lysed with 100 μL B-PER bacterial protein extraction reagent. Fluorescence emission intensities at 610 nm were then measured on a plate reader with excitation at 560 nm. Panel (A) shows the fluorescence of *p*BoF-containing mCherry expressed in DH10B (black) or C321.ΔA.exp (red) *E. coli* cells with different amounts of L-arabinose at 37 °C. Panel (B) shows the fluorescence of *p*BoF-containing mCherry expressed in C321.ΔA.exp, after varying the concentration of L-arabinose, induction temperature, and the length of induction period.

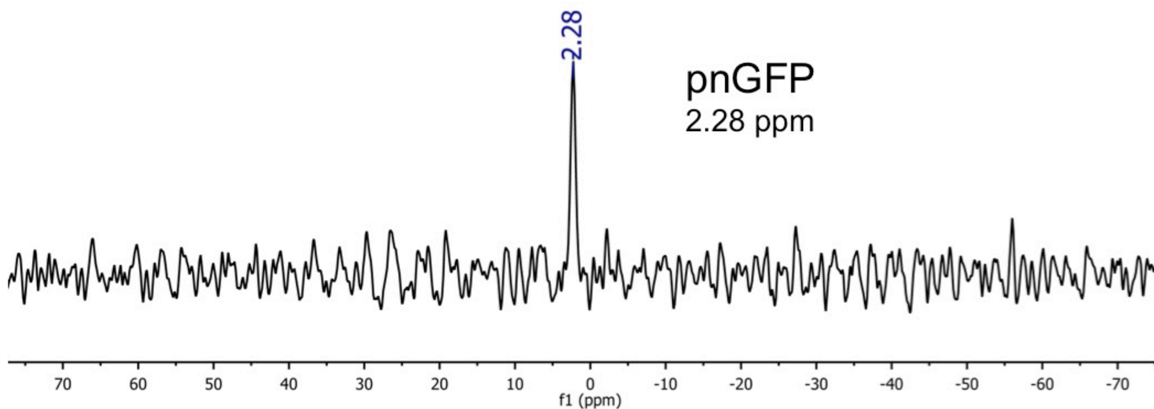

Figure S2. **^{11}B NMR spectra for pnGFP.**

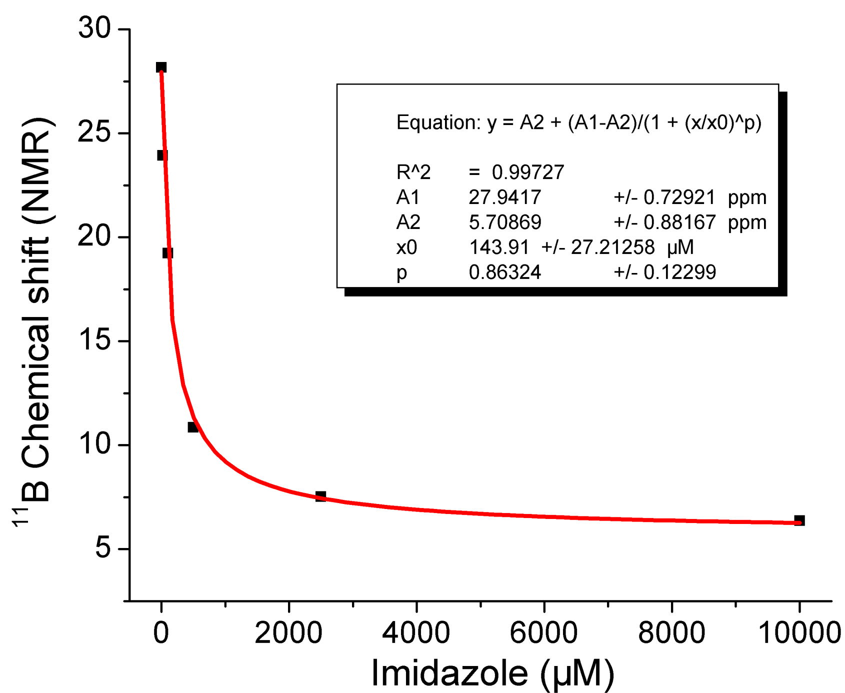

Figure S3. **^{11}B NMR chemical shifts for phenylboronic acid** (20 mM) in 20 mM phosphate buffer (pH 7.4, D_2O (v): H_2O (v) = 1:1), titrated with 0 mM, 20 mM, 100 mM, 500 mM, 2.5 M or 10 M imidazole. The data were fitted with the equation shown on the graph, which identified the extreme chemical shift for phenylboronic acid/imidazole complex to be 5.7 +/- 0.9 ppm.