

Diverse YqeK Ap4A hydrolases control biofilm formation in an Fe-dependent manner

Supporting Information

Chie Ueda^{1#}, *Natalie Chin*^{1#}, *Qianyi Yang*, *Luying Pan*, *Rheann Ponneah*², *Maria-Eirini Pandelia*^{1,*}

¹Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02453, United States

² Acton-Boxborough High School, Acton, Massachusetts 01720, United States

These authors contributed equally to this work

*Corresponding author: Maria-Eirini Pandelia

Email: mepandelia@brandeis.edu

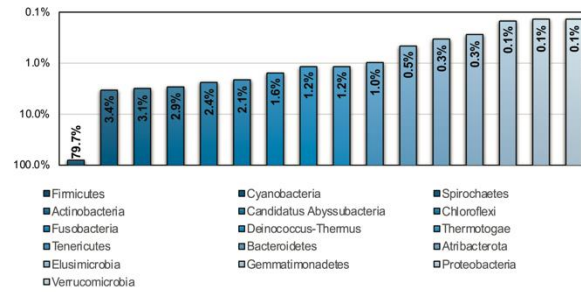


Figure S1. Percentage distribution of the major Phyla that are present in the SSN in the main text that consists of 8028 sequences.

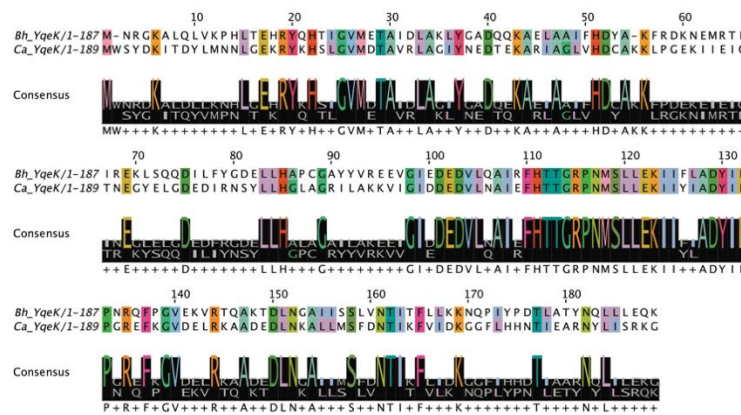


Figure S2. Sequence alignment of the *Ca* and *Bh* YqeK. Colored boxes represent sites that are 100% conserved among the two proteins, which share 42% sequence identity. Sequence alignment was generated with MAFFT[1].

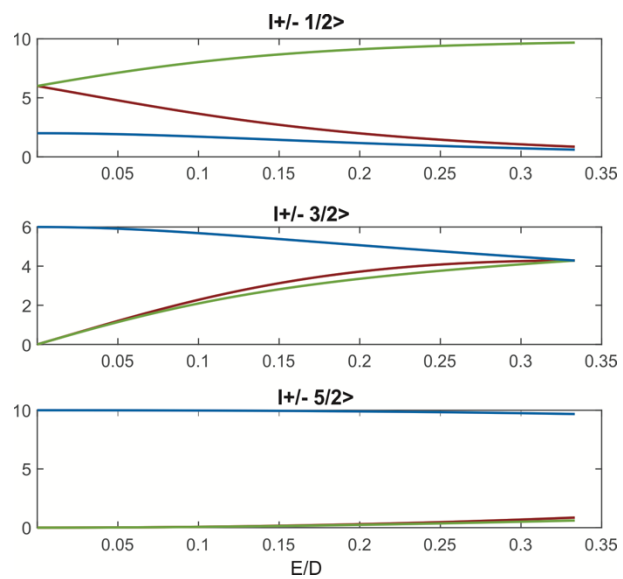


Figure S3. Representative rhombogram assuming a negative zero-field splitting parameter D for a system with an $S = 5/2$ similar to that observed in purple acid phosphatases [2, 3].

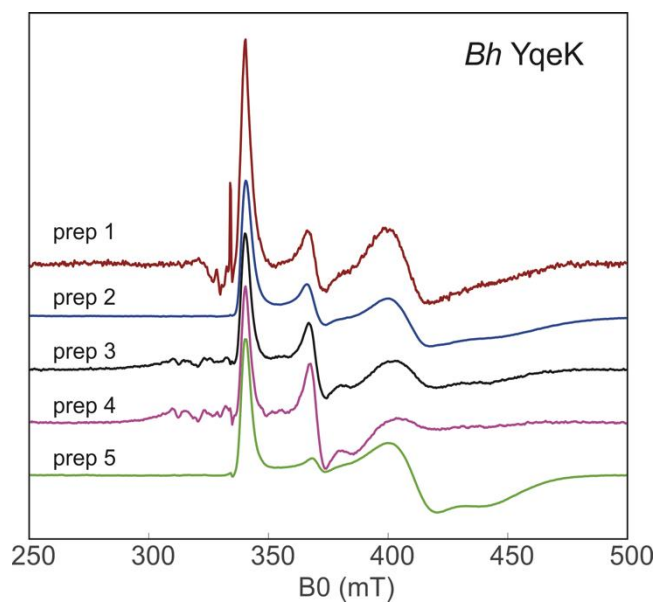


Figure S4. CW X-Band EPR spectra of different *Bh* YqeK preparations demonstrates a variability in the two mixed-valent species (i.e., Component 1, Component 2). All samples have been reduced with 2 molar excess of sodium ascorbate. Experimental conditions: temperature = 10 K, microwave frequency = 9.36 GHz, modulation amplitude = 1 mT, microwave power = 0.2 mW.

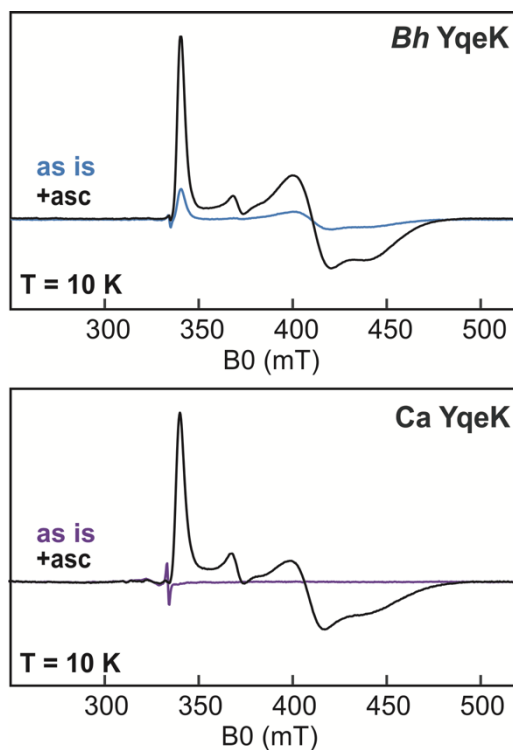


Figure S5. CW X-Band EPR spectra of the ascorbate-reduced M9-Fe *Bh* and *Ca*YqeK as isolated or reduced with 2 molar excess of sodium ascorbate. Experimental conditions: temperature = 10 K, microwave frequency = 9.36 GHz, modulation amplitude = 1 mT, microwave power = 0.2 mW.

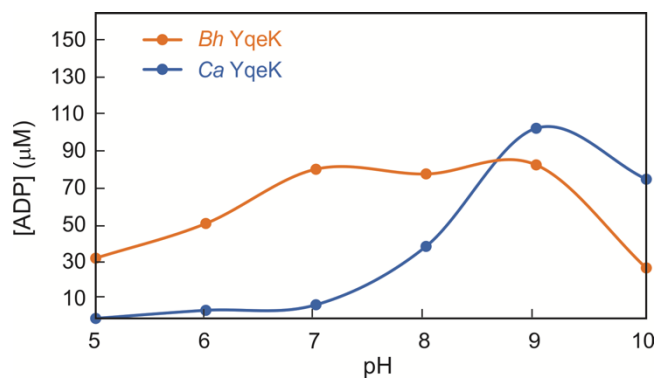


Figure S6. End-point activity assay to examine the pH dependence of the hydrolysis by *Bh* and *Ca* YqeK expressed in LB media. Experimental conditions: [YqeK] = 5 nM, [Ap4A] = 300 μM, reaction time 300 s under air.

Table S1. Primers utilized for the generation of the *Bh* YqeK variants.

Variant	Forward primer	Reverse primer
R18A	GACCGAACACGCATATCAACATACGATTGGC	AGATGCGGTTTCACCAGT
R133A	CGAACCGAACGCACAGTTCCCGG	ATGTAATCTGCCAGAAAG
K54A	TGATTACGCGGCATTCCGTGACAAAAATG	TGAAAAATGGCCGCCAGT

Table S2. Fe content of the variant *Bh* YqeK as estimated by ferrozine assays.

Variant	mol Fe/mol YqeK
R18A	1.54
K54A	1.62
R133A	1.81

REFERENCES

- [1]. Katoh, K.; Rozewicki, J.; Yamada, K. D., MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform* **2019**, *20* (4), 1160-1166.
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- [3]. Durmus, A.; Eicken, C.; Horst Sift, B.; Kratel, A.; Kappl, R.; Hüttermann, J.; Krebs, B., The active site of purple acid phosphatase from sweet potatoes (*Ipomoea batatas*). *Eur J Biochem* **1999**, *260* (3), 709-716.