

Engineered stable 5-hydroxymethylfurfural oxidase (HMFO) from *Methylovorus* sp. MP688 through B-factor analysis

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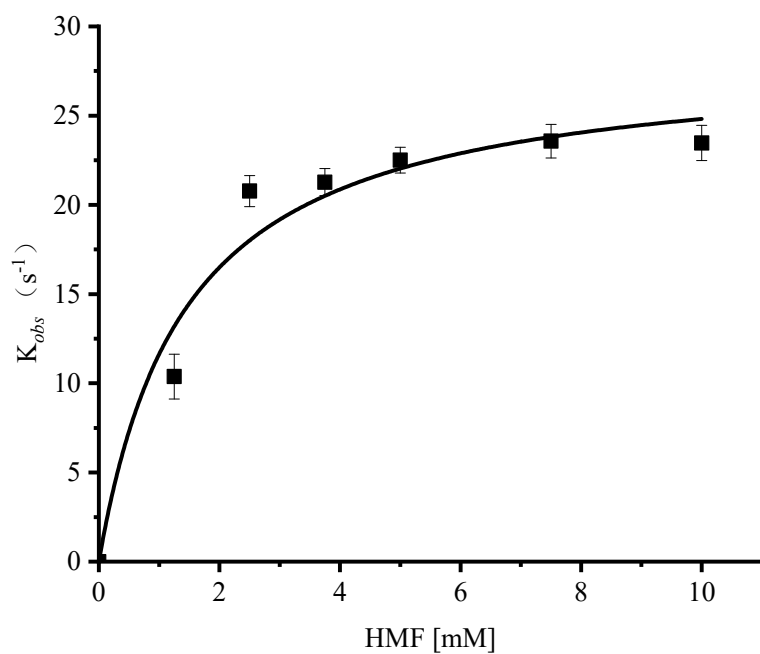
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Table S.1. List of the primers used for saturation mutagenesis

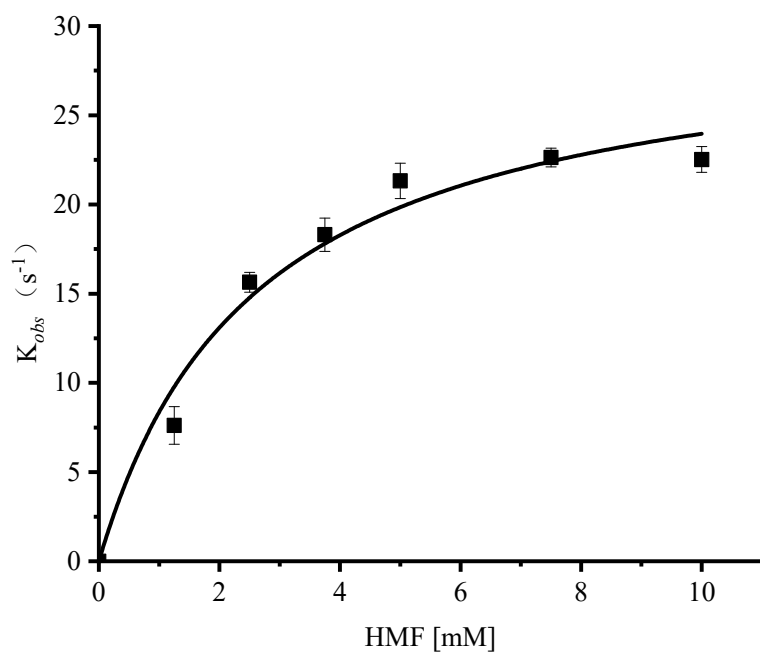
Mutational sites		Sequence
43	43-F	GATACCCCGNNKAACAATATTCCGCCGGAGATCCACGATGGC
	43-R	AATATTGTTMNNCGGGGTATCAATACCGGCCTCAATCAGCAA
44	44-F	ACCCCGGAANNKAATATTCCGCCGGAGATCCACGATGGCCTG
	44-R	CGGAATATTMNNTTCCGGGGTATCAATACCGGCCTCAATCAG
45	45-F	CCGGAACACNNKATTCCGCCGGAGATCCACGATGGCCTGCGC
	45-R	CGGCGGAATMNNGTTTTCCGGGGTATCAATACCGGCCTCAAT
81	81-F	GCGGAACACCCGNNKATCACGCGCGAGCCGCAGTTCTATGAACAA
	81-R	CGCGTGATMNNCGGGTGTTCCGCGGCACGGTAGACGGTCA
82	82-F	GAACACCCGGGTNNKACGCGCGAGCCGCAGTTCTATGAACAAGGC
	82-R	CTCGCGCGTMNNAACCGGGTGTTCCGCGGCACGGTAGACG
252	252-F	CTGCGTGGTNNKGAAACCCTGCAAGTTCAAGCGCGCGAGGTC
	252-R	CAGGGTTTCMNNACCACGCAGCACGCTCACACCGGTGATTGC
253	253-F	GCGTGGTCGCNNKACCCTGCAAGTTCAAGCGCGCGAGGTCATC
	253-R	GCAGGGTMNNGCGACCACGCAGCACGCTCACACCGGTGATT
319	319-F	CTGACCGAGNNKGCACGTGCAGACGCCAGCACGGGTAAAG
	319-R	TGCACGTGCMNNCTCGGTCAGCGGGGCAACCACACCGATA
321	321-F	TGACCGAGCAGGCANNKGCAGACGCCAGCACGGGTAAAGCCG
	321-R	TGGCGTCTGCMNNTGCCTGCTCGGTTCAGCGGGGCAACCACAC
328	328-F	CAGCACGGGTNNKGCCGGCTCTCGCCATCAACTGGGTATCC
	328-R	GAGAGCCGGCMNNAACCGTGCTGGCGTCTGCACGTGCCTGCT



Michaelis-Menten

Best-fit values		8B-HMFO
K_{cat}		28.39
K_m		3.49
Std. Error		
K_{cat}		2.47
K_m		0.48
95% Confidence Intervals		
K_{cat}		25.92 to 30.86
K_m		0.96 to 1.93
R^2		0.96

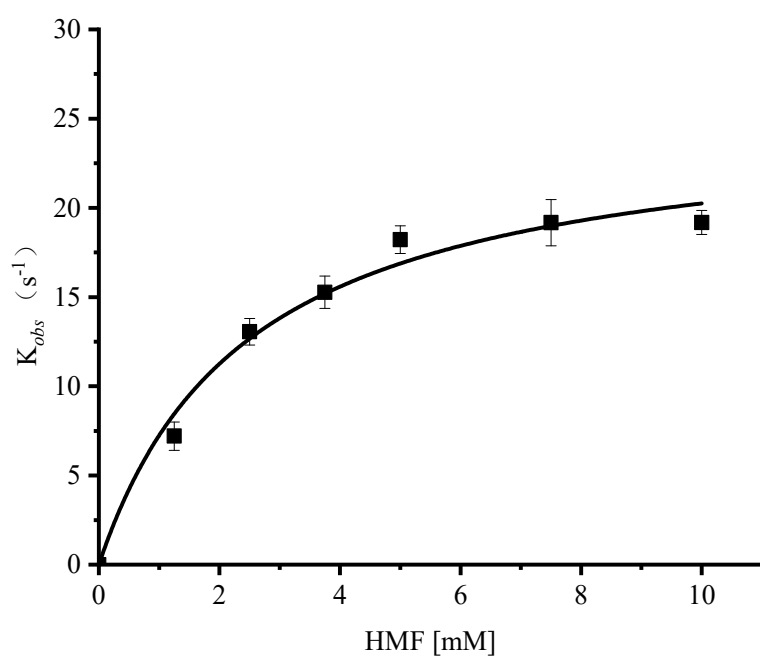
Figure S.1. Michaelis-Menten curve for the HMF catalyzed by 8BxHFMO.



Michaelis-Menten

Best-fit values		N44G-HMFO
K_{cat}		30.23
K_m		2.62
Std. Error		
K_{cat}		2.65
K_m		0.65
95% Confidence Intervals		
K_{cat}		27.58 to 32.87
K_m		1.96 to 3.27
R^2		0.98

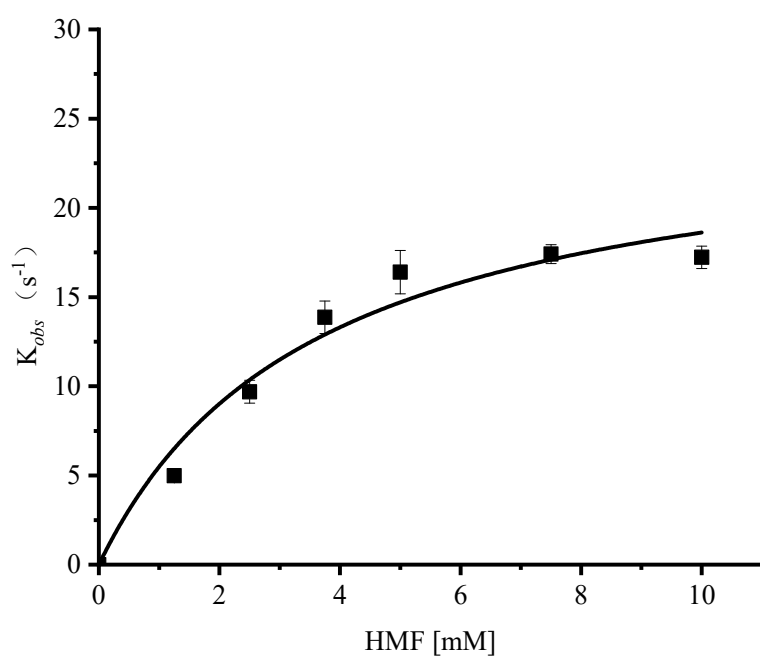
Figure S.2. Michaelis-Menten curve for the HMF catalyzed by Q319K.



Michaelis-Menten

Best-fit values		Q319K-HMFO
K_{cat}		25.30
K_m		2.49
Std. Error		
K_{cat}		1.75
K_m		0.50
95% Confidence Intervals		
K_{cat}		23.55 to 27.05
K_m		1.99 to 3.00
R^2		0.98

Figure S.3. Michaelis-Menten curve for the HMF catalyzed by N44G.



Michaelis-Menten

Best-fit values

N44G-Q319K-HMFO

K_{cat} 25.36

K_m 3.62

Std. Error

K_{cat} 3.18

K_m 1.11

95% Confidence Intervals

K_{cat} 22.18 to 28.53

K_m 2.51 to 4.73

R^2 0.97

Figure S.4. Michaelis-Menten curve for the HMF catalyzed by N44G-Q319K.