Supporting Information

Structural basis and binding kinetics of vaborbactam in class A β-lactamase inhibition

Orville A. Pemberton[a], Ruslan Tsivkovski[b], Maxim Totrov[c], Olga Lomovskaya[b#], and Yu Chen[a#]

[a]Department of Molecular Medicine, University of South Florida Morsani College of Medicine, Tampa, Florida, USA

[b]Qpex Biopharma, Inc., San Diego, California, USA

[c]Molsoft L.L.C., San Diego, California, USA

#Address correspondence to Olga Lomovskaya, olomovskaya@qpexbio.com; Yu Chen, ychen1@health.usf.edu

Table S1. X-ray crystallographic statistics for CTX-M-14 and KPC-2 with vaborbactam

	CTX-M-14:Vaborbactam	KPC-2:Vaborbactam
PDB code	6V7H	6V7I
Data collection		
Space group	P 1 2_1 1	P 2 2_1 2_1
Cell dimensions		
a, *b*, *c* (Å)	45.30, 106.70, 47.86	55.77, 59.97, 77.72
a, b, c (°)	90.00, 101.64, 90.00	90.00, 90.00, 90.00
Wavelength (Å)	0.9184	0.9791
Resolution (Å)	53.36–1.00 (1.05–1.00)	47.48–1.25 (1.32–1.25)
No. of unique reflections	228,516 (30,884)	72,556 (10,447)
$\langle I \rangle / \sigma \langle I \rangle$	13.3 (5.5)	8.9 (1.8)
Completeness (%)	95.7 (88.6)	99.7 (99.7)
Redundancy	3.6 (2.8)	5.8 (5.8)
R_{merge}	0.062 (0.198)	0.090 (0.912)
$CC_{1/2}$	0.997 (0.939)	0.998 (0.752)
Refinement		
Resolution (Å)	46.88–1.00 (1.05–1.00)	47.48–1.25 (1.32–1.25)
No. of reflections	228,425 (20,382)	72,494 (7,158)
R_{work}/R_{free}	0.1046/0.1209	0.1566/0.1789
No. of non-hydrogen atoms	5,322	2,526
Protein	4,136	2,160
Ligands	139	66
Solvent	1,047	300
B-factors (Å^2)		
Protein	7.31	15.07
Ligands	13.43	28.83
Solvent	25.12	31.37
RMSD		
Bond length (Å)	0.006	0.009
Bond angle (°)	1.10	1.08
Ramachandran		
Favored (%)	97.10	98.88
Allowed (%)	2.51	1.12
Outliers (%)	0.39	0.00

*Values in parentheses are for highest-resolution shell.

Table S2. The effect of KPC-2 W105 mutations on antibiotic susceptibility in an efflux deficient strain of *P. aeruginosa* as measured by MIC (μg/mL)

Strain		Aztreonam	Ceftazidime	Piperacillin	Meropenem
PAM4175	vector	0.125	0.25	0.125	0.125
PAM4135	WT	128	16	128	32
PAM4576	W105F	32	4	64	32
PAM4577	W105N	>256	32	32	16
PAM4578	W105L	64	1	8	8
PAM4579	W105V	64	1	16	8
PAM4618	W105D	2	0.5	1	32
PAM4613	W105A	128	4	32	8
PAM4614	W105Y	64	8	64	32
PAM4616	W105S	256	8	32	8

Table S3. The effect of KPC-2 S130G mutation on antibiotic susceptibility in an efflux deficient strain of *P. aeruginosa* as measured by MIC (µg/mL)

Strain	KPC-2	Aztreonam	Ceftazidime	Cefepime	Carbenicillin	Piperacillin	Meropenem	Imipenem
PAM4175	vector	0.125	0.25	0.125	0.5	0.125	0.125	1
PAM4135	WT	256	32	128	1024	128	128	16
PAM4648	S130G	0.25	1	0.125	128	64	0.25	1

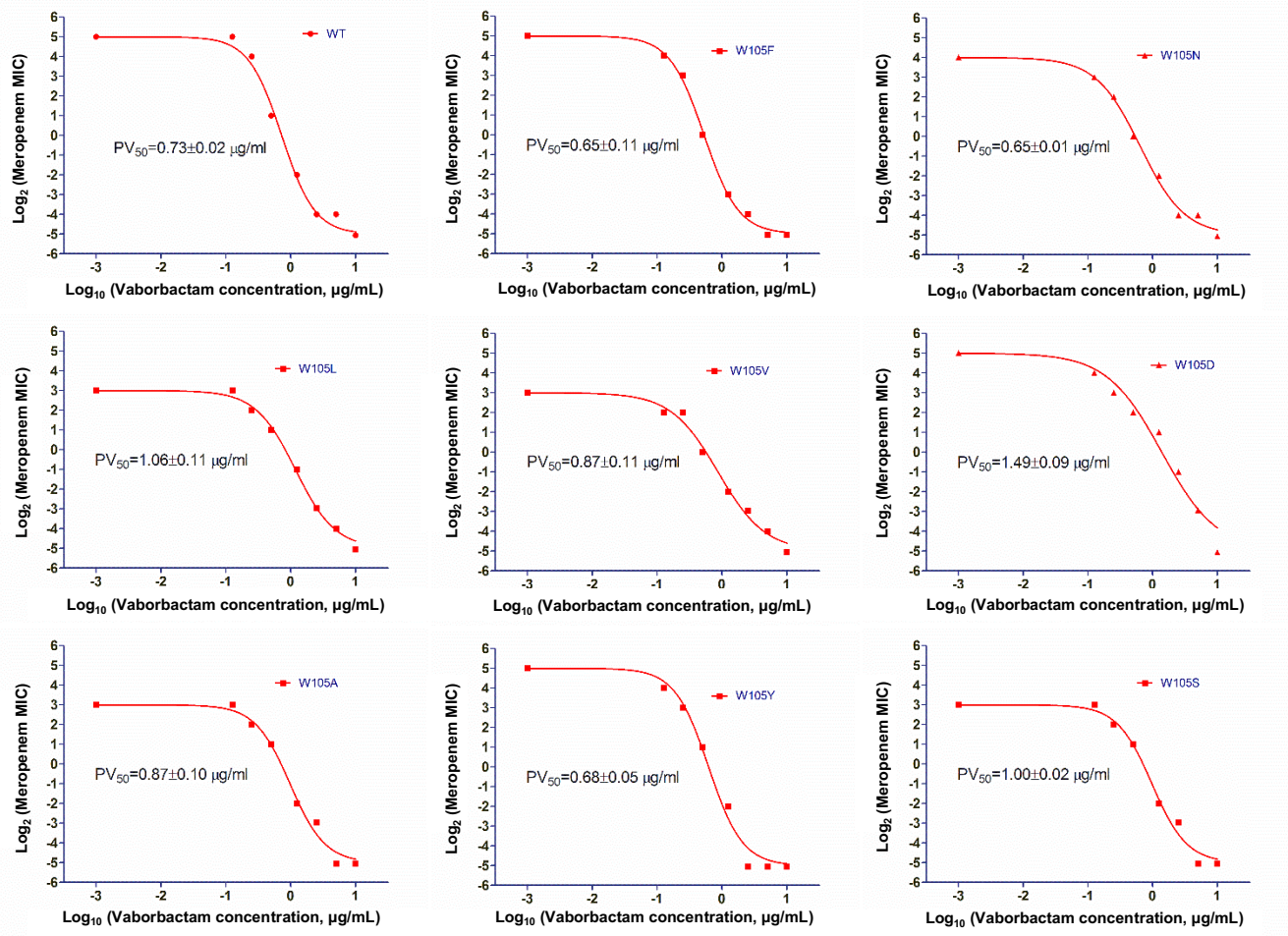

Figure S1. **Effect of W105 mutations in KPC-2 on the potentiation of meropenem by vaborbactam against KPC-2 producing *P. aeruginosa***. PV_{50} (PV, potentiation value) is the minimal concentration of vaborbactam that is required to reduce meropenem's MIC to the middle of the MIC range where the highest MIC is the MIC for KPC-2 producing strain with no vaborbactam added and the lowest MIC is the MIC for the vector only strain, corresponding to the complete inhibition of KPC-2.

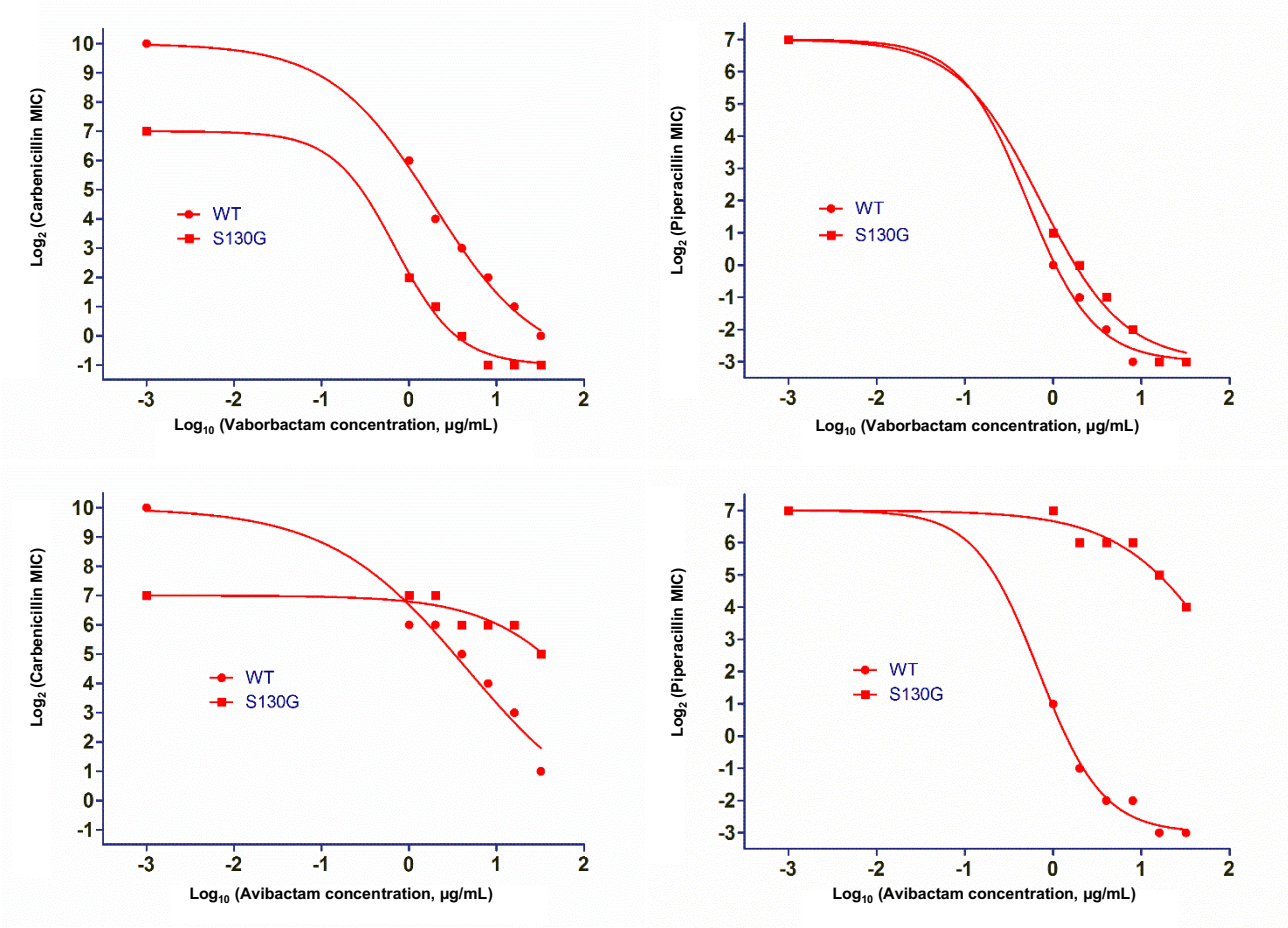

Figure S2. Effect of S130G mutation in KPC-2 on the potentiation of carbenicillin and piperacillin by vaborbactam and avibactam against KPC-2 producing *P. aeruginosa*.

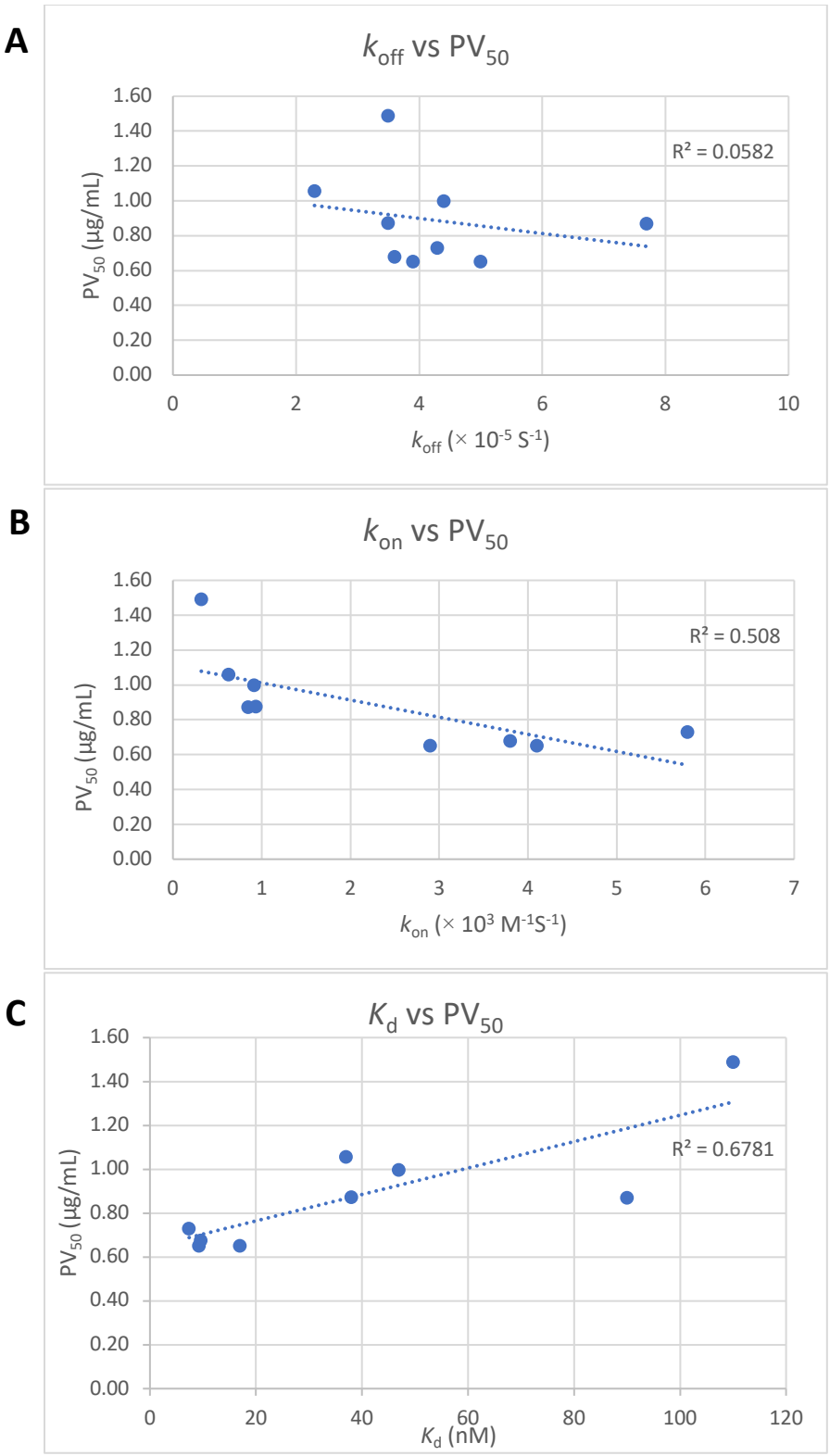

Figure S3. Graph of vaborbactam k_{off}, k_{on}, and K_d versus vaborbactam potency (PV_{50}) of meropenem potentiation for W105 mutations in KPC-2.