



Supplementary Materials: Phage Anti-Pycsar Proteins Efficiently Degrade β -Lactam Antibiotics

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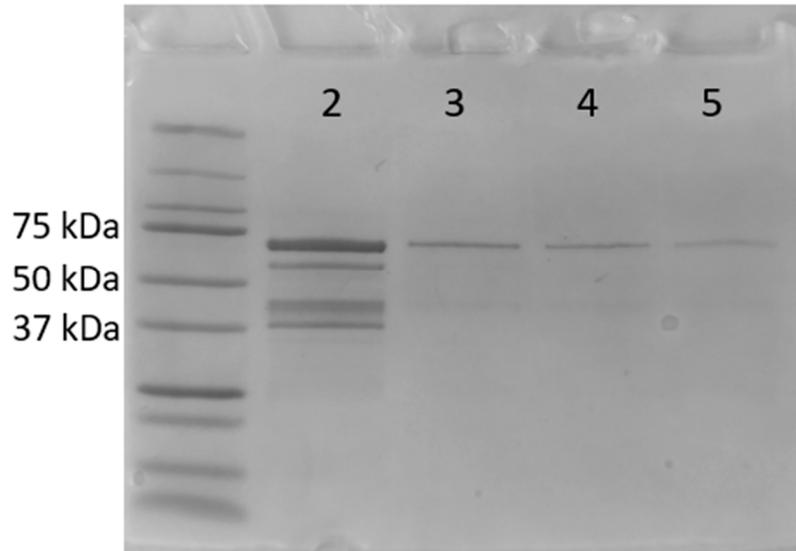


Figure S1. SDS-PAGE of the purification of MBP-tagged Apyc_{Goe3} using maltose binding affinity chromatography. The calculated molecular weight of Apyc_{Goe3} is 74.5 kDa. Fractions 3 to 5 were combined and concentrated for future experiments.

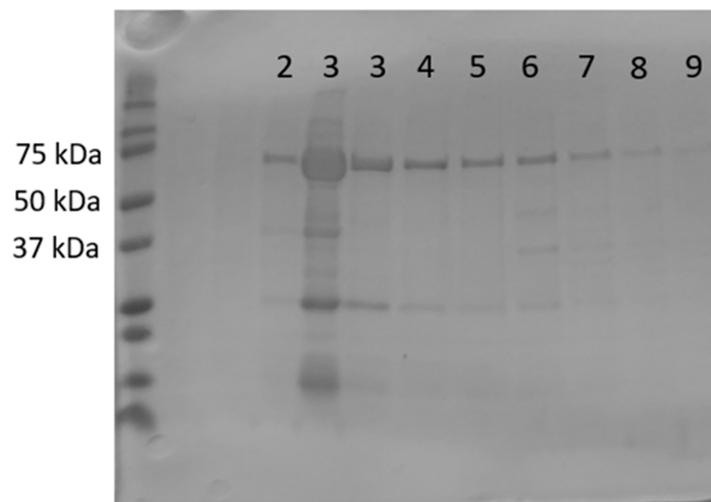


Figure S2. SDS-PAGE of the purification of MBP-tagged Apyc_{Goe3}(D178S) using maltose binding affinity chromatography. The calculated molecular weight of Apyc_{Goe3}(D178S) is 74.5 kDa. Fractions 4 and 5 were concentrated for future experiments.

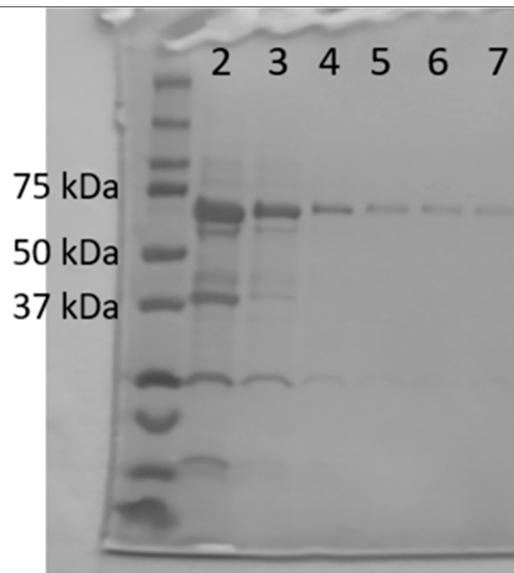


Figure S3. SDS-PAGE of the purification of MBP-tagged ApyCGrass using maltose binding affinity chromatography. The calculated molecular weight of ApyCGrass is 69.2 kDa. Fractions 3 to 7 were combined and concentrated for future experiments.

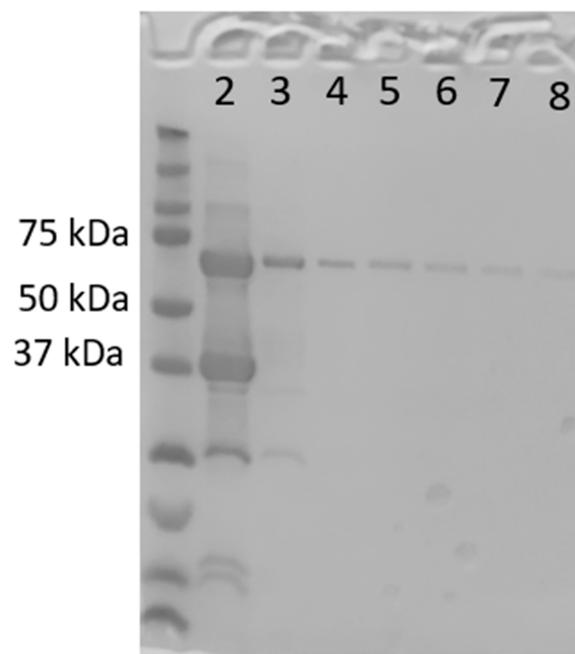


Figure S4. SDS-PAGE of the purification of MBP-tagged ApyCGrass (D161S) using maltose binding affinity chromatography. The calculated molecular weight of ApyCGrass (D161S) is 69.2 kDa. Fractions 3 to 8 were combined and concentrated for future experiments.

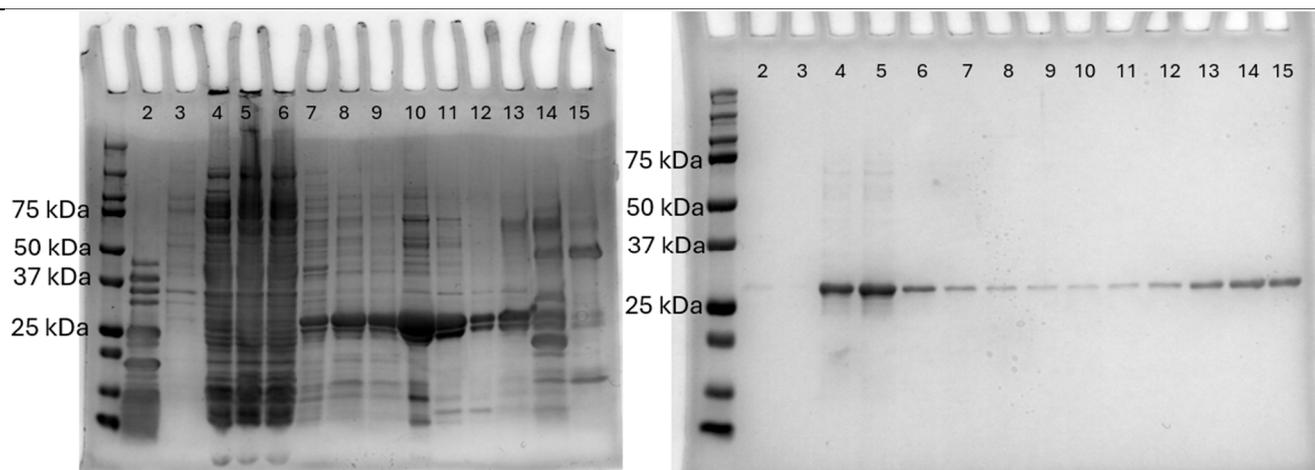


Figure S5. (Left) SDS-PAGE of the purification of hexahistidine-tagged Apyc^{Goe3} using immobilised metal affinity chromatography and (Right) SDS-PAGE of fractions 7 to 13 after being concentrated and run through size exclusion chromatography. The calculated molecular weight of hexahistidine-tagged Apyc^{Goe3} is 32 kDa.

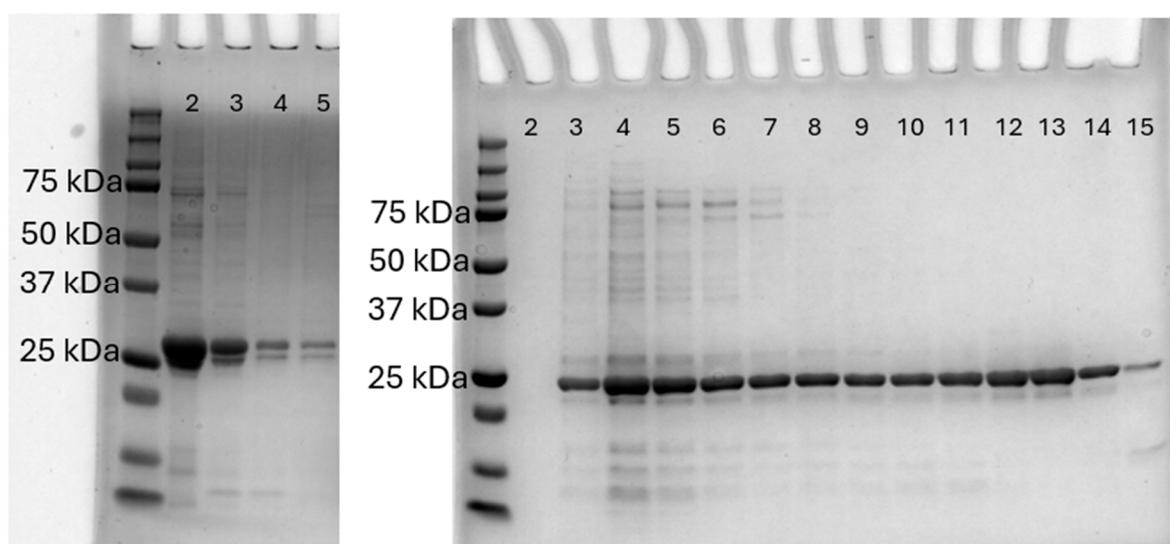


Figure S6. (Left) SDS-PAGE of the purification of hexahistidine-tagged Apyc^{Grass} using immobilised metal affinity chromatography and (Right) SDS-PAGE of fractions 2 to 5 after being concentrated and run through size exclusion chromatography. The calculated molecular weight of hexahistidine tagged Apyc^{Goe3} is 26.7 kDa.

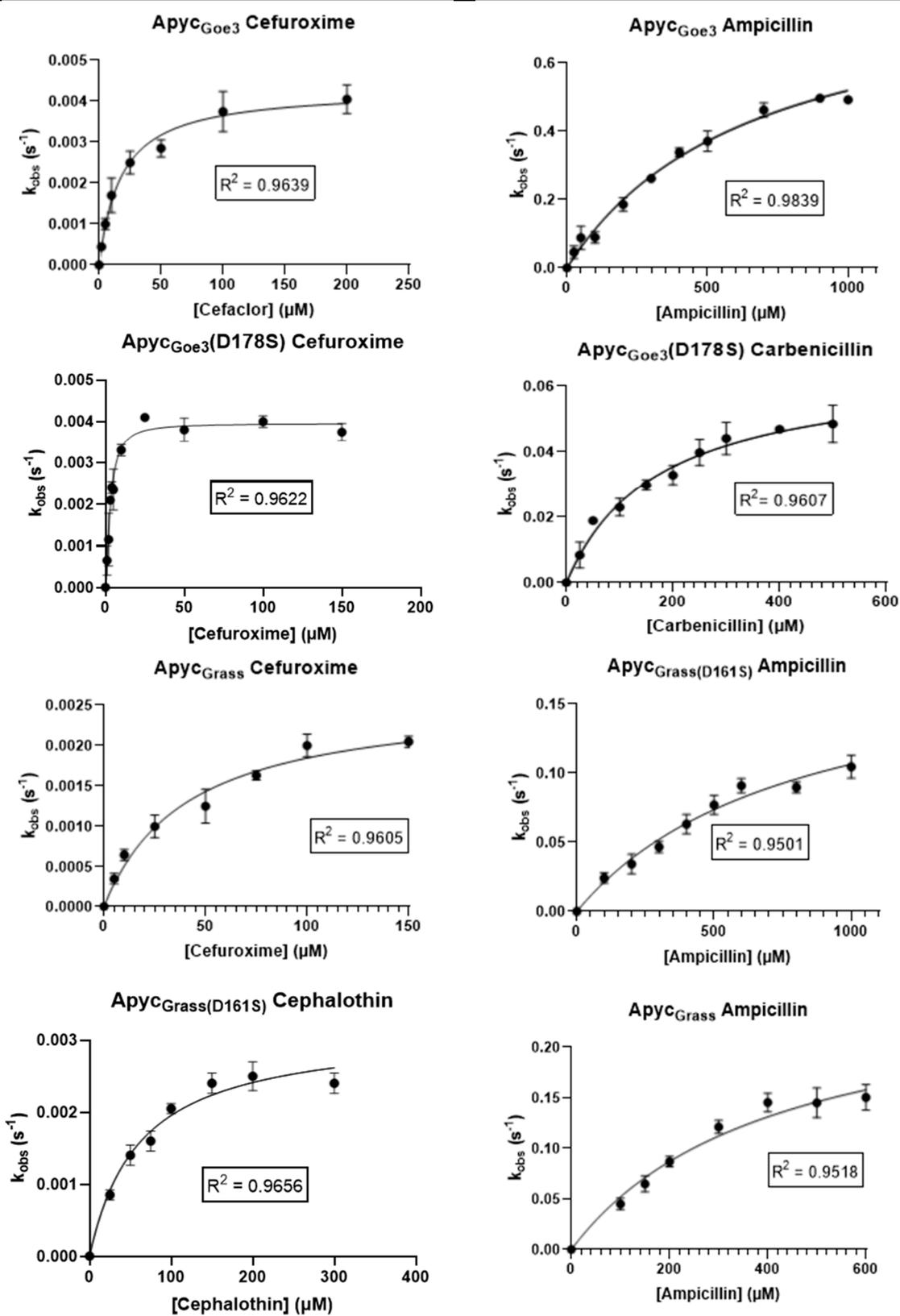


Figure S7. Representative Michaelis-Menten curves for reactions catalysed by Apyc_{Goe3}, Apyc_{Goe3}(D178S), Apyc_{Grass} and Apyc_{Grass}(D161S) with relevant substrates.



	64	66	68	69	...	154	...	178	...	233
Apyc _{Goe3}	H	T	H	A	D	H		D		H
Apyc _{Grass}	H	T	H	A	D	H		D		H
Apyc1	H	T	H	A	D	H		D		H
PNGM-1	H	L	H	T	D	H		D		H
TupBlac	H	L	H	N	D	H		D		H
ZipD	H	L	H	G	D	H		D		H
AIM-1	H	E	H	F	D	H		S		H

Figure S8. Aligned metal-binding residues of Apyc orthologs and representatives of the MBL-fold superfamily. Amino acid sequences from various functional subgroups of the MBL-fold superfamily were aligned using structure-guided sequence alignment with MAFFT-DASH (L-INS-i) [55,56]. Columns corresponding to residues comprising the α (H64, H66, H154) and β (D68, H69, H233) metal-binding sites, and the metal-bridging position (178) were extracted for representative sequences. Apyc_{Goe3} and Apyc_{Grass} display the same metal binding motifs as Apyc1 and much of the broader superfamily, and possess the bridging aspartate residue absent in true metallo- β -lactamases (*i.e.*, the B1, B2, and B3 MBLs).



Table S1. Catalytic parameters of representative members of each Ambler class of β -lactamases. Classes A, C, and D are SBLs, while Class B (inclusive of the B1, B2, and B3 subgroups) are MBLs. Units of k_{cat} , K_M , and k_{cat}/K_M are s^{-1} , μM , and $s^{-1} mM^{-1}$. ^aYong *et al.* [37], ^bBottoni *et al.* [58], ^cHorsfall *et al.* [59], ^dSegatore *et al.* [60], ^eBebrone *et al.* [61], ^fVenkatachalam *et al.* [62], ^gDe Wals *et al.* [63], ^hPoirel *et al.* [64], ⁱRobin *et al.* [65], ^jMarcocchia *et al.* [66], ^hChiou *et al.* [67], ⁱMammeri *et al.* [68], ^jMazzariol *et al.* [69], ^kLenfant *et al.* [70]. N.H. - no hydrolytic activity detected. | N.D. - not determined.

Substrate	TEM-1 (Class A)			NDM-1 ^a (Class B1)			CphA (Class B2)			AIM-1 ^c (Class B3)			AmpC (<i>E. coli</i> K12) (Class C)			OXA-48 (Class D)		
	k_{cat}	K_M	k_{cat}/K_M M	k_{cat}	K_M	k_{cat}/K_M M	k_{cat}	K_M	k_{cat}/K_M M	k_{cat}	K_M	k_{cat}/K_M M	k_{cat}	K_M	k_{cat}/K_M M	k_{cat}	K_M	k_{cat}/K_M M
Penicillins																		
Penicillin G	1660 ^g	62 ^g	26,77 4 ^g	11	16	680	3 ^d / 0.03 ^e	630 ^d / 870 ^e	4.8 ^d / 0.034 ^e	778	31	25,00 0	45	4.4	10,22 7	245 ^h	40 ^h	6100 ^h
Ampicillin	1450 ^f	71 ^f	20,42 2 ^f	15	22	660	<0.01 ^e	2500 ^e	<0.00 4	594	41	14,00 0	4.2	3.5	1200	340 ^h	5200 ^h	65 ^h
Carbenicillin	120 ^k	14 ^k	8571 ^k	108 ⁱ	285 ^j	379 ^j	10 ^d	500 ^d	20 ^d	-	-	-	0.002 ^j	0.1 ^j	20 ^j	311 ^k	57 ^k	5456 ^k
Carbapenems																		
Meropenem	N.H.	N.H.	N.H.	12	49	250	3100 ^b /1600 ^b /1940 ^b / 53 ^d	250 ^d	212 ^d	1000	163	6100	-	-	-	0.1 ^h	200 ^h	0.5 ^h
Imipenem	N.H.	N.H.	N.H.	20	95	210	460 ^b / 140 ^d / 1200 ^e	110 ^b / 86 ^d / 340 ^e	4180 ^b / 1627 ^d / / 3529 ^e	1700	97	18,00 0	0.09 ⁱ	32,00 0 ⁱ	0.002 8 ⁱ	2 ^h	14 ^h	145 ^h
Cephalosporins																		
Cephalothin	77 ^g	180 ^g	427 ^g	4	10	400	-	-	-	529	38	14,00 0	300	42	7142	3 ^h	120 ^h	150 ^h
Cefuroxime	<0.1 ⁱ	N.D ⁱ	N.D ⁱ	5	8	610	-	-	-	292	29	10,00 0	0.15 ^j	0.15 ^j	1000 ^j	-	-	-