SUPPORTING INFORMATION

A two-component protease in *Methylorubrum extorquens* with high activity toward the peptide precursor of the redox cofactor pyrroloquinoline quinone

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Class/phylum/order	Genus	Species	
α -proteobacteria	30	41	
β -proteobacteria	18	30	
γ-proteobacteria	23	45	
ɛ-proteobacteria	1	1	
Actinobacteria	5	6	
Verrucomicrobia	2	2	
Acidobacteriia	1	1	
Aquificales	1	1	

Table S1. Summary of the bacterial genera and species studied in Shen *et al.* (1).

Table S2. Bacterial species analyzed for Pqq-encoding genes by Shen and co-workers (1). The Excel Table shows the bacterial species, grouped by phylum and class (in the case of the phylum Proteobacteria), the PQQ production of each strain (according to (2)), the gene arrangement from the analysis of Shen *et al.* (1), the gene arrangement found in this analysis the PqqF and PqqG definitions, GenBank accession numbers, number of amino acid residues, zinc-binding consensus sequences (for PqqF) and whether the genes encoding PqqF and PqqG are contiguous and the location concerning the *pqq* operon.



Figure S1. SDS-PAGE gels of purified, his-tagged and untagged PqqF and PqqG. The lanes are as follows: (1), (4), (7) protein ladder (10 to 200 KDa), (2) His₆PqqF, (3) PqqF, (5) His₆PqqG and (6) PqqG. The molecular weights predicted from the protein sequences are: His₆PqqF 53.14 kDa, PqqF 51.26 kDa, His₆PqqG 47.90 kDa, PqqG 46.01 kDa. Gels #1, #2 and #3 were obtained in different experiments. Gel #1 shows the ladder lane separated from the protein sample lanes.



FIGURE S2. Binding of PqqF and PqqG observed by SPR. Representative datasets of each experiment are shown. (*A*) His₆PqqG was used as a ligand and PqqF was used as analyte, (*B*) His₆PqqF was used as a ligand and PqqF was used as analyte, (*B*) His₆PqqF was used as a ligand and PqqG as used as analyte. In both cases the SPR response increased as the analyte concentration increased, from 45 nM to 9 μ M. However, both PqqF and PqqG also showed non-specific adsorption to the SPR chip, leading us to perform K_d measurements by ITC. (see Fig. 4 in main text).



FIGURE S3. The most usual PQQ gene arrangements in the genome of *(top panel)* γ -proteobacteria and *(bottom panel)* α -proteobacteria.

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consensus EAA++EVTEETLI DIGLI DVV/LEDHBAPVVTH/WWWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWWSSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWS/GSADEP+GKSGLAHELEHL/MEKGTKKHEPGEESK TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWS/GSADEP+GKSGLAHELEHL/MEKGTK/HEPGEESK TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWS/GSADEP+GKSGLAHELEHL/MEKGTK/HEPGEESK/TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWS/GSADEP+GKSGLAHELEHL/MEKGTK/HEPGEESK/TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEDHBAPVVTH/WWWS/GSADEP+GKSGLAHELEHL/MEKGTK/HEPGEESK/TVAAL GGOENAETSVD/TA/EGRVAK/RL ELEMMELEADBMANLVL TEEVAPERDVV/LEERDWSAL/GK/HEPGE/GKSGLAHELE/H/MEKGTK/HEPGE/GKSGLAHELE/H/MEKGTK/HEPGE/GKSGLAHELE/H/MEKGTK/HEPGE/GKSGLAHELE/H/MEKGTK/HEPGE/GKSGLAHELE/H/MEKGTK/HEPGE/GKSGLAHELE/H/MEKGTK/HEPGE/GKSGLAHELE/H/MEKGTK/HEPGE/GKSGLAHELE/H/MEKGTK/HEPGE/GKSGLAHELE/H/MEKGTK/HEFGK/HEFGK/H		VMAKVHEFTLKNGLKL	LVKEDPRAPVMVSQVVVKV	GSSYEYNGITG	SHMLEHMM	FKGTKNLEPNQFSQIISAN	GGEENAFTGRDYT	AYFEQMANDQVEVSFRL	ADRMRNLVLIPEELRKE	KQVVMEERRM	RTEDNPNALTYERFN	IATAFLSGPYHHP	VIGNMSE
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Conservation 00523142263*2*9599*45*3*7+775389+695*855921*28*98*5*99*+4+134227499339535*47*5*72*76*786838649235837*8*9326372234724*55*96*664*7464725461*223+6593345+33*79*9238 quality consensus EAA++EVTEET I DNGI DVVVI EDHRAPVVTHMWWWWGSADEP+GKSG I AHEI EHI MEKGTKKHPPGEESKTVAAI GGGENAETSYDYTAYEORVAKDRI EI MMEL EADRMANI VI TDEEVAPERDVVI EERPMRTEDDPSAL I SECMMAAI EI NHP YGRP I I GAMHE	aangamiatian	in a stational state	والمكالة ومعادل المرا	ana tan		L . L L .	a shekara	a de la sela de la	l na ad		Las Las		
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quality consensus EAR++EVTEETI DIGI DVVV IEDHRAPVVTHMVWVVGSADEP+GKSG LAHEI EHI MEKGTKKHEPGEESKTVALI GGGENAETSYDYTAYEORVAKDRI EI MMEI EADRMANI VI TDEEVAPERDVVI EERPMRTEDDPSALI SECMMAALEI NHP YGRP I I GMMHE													.5 5235
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Figure S4. Segment of the sequence alignment of the N-terminal of PqqF (M16B peptidases) from α -proteobacteria. Highlighted in red is the zincbinding consensus HxLEH. Sequences were aligned using Clustal Omega and the figure was generated and analyzed in Jalview (3).

HxxEH

	90	100	110	120	130	140	150		160	170	180	190	200	210	220
	3VRFGTLPNGMRFA		VM <mark>H</mark> NA	TPSGQVA-		IRFRIAT	SLQ <mark>E</mark> N-DDQ		FLEHMA	KGSTHVPE	GEMIRTLQ-	RLGLAFGPDT	IAST GYNE	TVÝALDLÞEAKF	DTVST <mark>GL</mark> MLMRETAS
	(YLFKE <mark>L</mark> S <mark>NGLRV</mark> L		VVKTD'	Y-PDLVS-		VQIP <mark>V</mark> SV <mark>G</mark>	SRDEDEAGK	TGFAH	IFFEHMM	-K <mark>gs</mark> dkfpg	DVYSDLFK-	- NAG VDN		NYHLDFSKD	- HLDKVLE I QADHFK
	VIEEYKLDNGFRVI		<mark>L</mark> APNEI	K-ENKVY-		VNTVYLT <mark>G</mark>	SLND <mark>P</mark> -KGK	GGL <mark>A</mark> H	ILLEHLA	KGTVNVKG	DEFQRRLD-	- QYT LMT	IASTDYYS	T <mark>kyiniv</mark> rpekn	- ALNE ILYLEAERMD
	IVEEYKLDNGFRVV		<mark>L</mark> APNDI	K-ENKIF-		INTIYLT <mark>G</mark>	SLND <mark>P</mark> - QGK	SGLAF	ILLEHLA	- K <mark>g</mark> tqnvkg	EEFQRRLD-	- QYT LMT	IASTDYYS	T <mark>KYTN I V</mark> RPEKI	- ALDQVLYLESERMD
	IIEEYK <mark>L</mark> DNGFRVV		<mark>L</mark> APNDI	K-ENKIF-		INTIYLT <mark>G</mark>	SLND <mark>P</mark> - QGK	G <mark>GL </mark> AH	ILLEHLA	- K <mark>g</mark> tanvke	EEFQRRLD-	- QYT LMT	IASTDYYS	T <mark>KYTN I V</mark> RPEKI	- ALDQVLYLESERMD
	3ITEHRLPNGLRIV		<mark>L</mark> APDA.	A-KATTT-		VNITYLV <mark>G</mark>	SRHEN - YGE	TGM <mark>A</mark> H	ILLEHL	KGTPSLPG	KTIPAEFA-	-RR <mark>G</mark> MSV	IGTTAQDR	TNYFETFTASDE) - NLDWALRMEADRMV
	3ITEYR <mark>L</mark> PNGLRIV		<mark>L</mark> APDA.	4-KATTT-		VNTTYLV <mark>G</mark>	SRHEN - YGE	TGM <mark>A</mark> H	ILLEHL	-K <mark>g</mark> tpslpg	KTIPTEFA-	-RR <mark>G</mark> MSVN	IGTTAQDR	TNY <mark>F</mark> GTFSANDE	E-NLDWALRMEADRMV
	3TEYR <mark>L</mark> TNGLQVL		<mark>L</mark> VPDA:	S-KPTTT-		VNLTYHV <mark>G</mark>	SRHEN - YGE	TGM <mark>A</mark> ⊢	ILLEHLM	-K <mark>g</mark> tpttpn	WVGEFT-	- KR <mark>G</mark> LRA	IGS TWFDR	TNY <mark>F</mark> ASFA <mark>A</mark> NDE) - NLRWELSWHADAMV
	21 DQEE <mark>LPNGL</mark> TVL		VDPFH	Q- <mark>A</mark> DVVS-		LQFWCAT <mark>G</mark>	S I HEGKYAG	SGI₿⊢	ILLEHL	-KGTDKRKG	NQIAWEMQ-	- SL <mark>G</mark> <mark>G</mark> HL	IAYTTYNR	VYHVDLPST	- HWKEALEILADIVF
	[VTRATLSNGLRV]		VVRDT	<mark>A</mark> PVVQ-		TMLNYET <mark>G</mark>	SVNA <mark>P</mark> - KGF	PGTAH	IALEHM <mark>M</mark> I	-NGSQTLSF	DQLSTISA-	- QL <mark>G</mark> NND	IADTTSDV	TQYYFKAPAS	- DLDVLLRIEAGRMR
	2VVRATLPNGLKVV		I VPNR	<mark>A</mark> PVVT -		TE INYLV <mark>G</mark>	SAEV <mark>P</mark> -EGF	PGT <mark>A</mark> H	IALEHMMI	-R <mark>G</mark> SKGLDK	.DQLAAIGT -	-RLGGSYN	IADTTEDV	TQYFYTAQAQ	- DLPVLLKIEALRMN
	AVRATLPNGLRVV		V I RDR	APVVT-		TE INYLVG	ASEAP - KGF	PGTAH	IALEHMMI	RGSAGLDK	DQLAAIGA-	-RLGGSY	IADTTENV	TQYFYTAPAE	- DLGVMLRIEALRMR
	APVRTTLPNGMR11		I VPDR	APVVT-		TEMNYLV <mark>G</mark>	SAAAP - RGF	PGTAF	IALEHMMI	RGSKGLDK	DQLAAIGA-	- RMG GNY	ADTTEST	IQFFYTAPAE	-DLDIALRIEALRMN
	AYTRLTLPNGLTVV		VHEDHI	K-APVVA-		VS IWYH I G	SGDEP - AGK	TGFIAF	ILFEHLMI	SGSENNKG	SFFAPLEK-	- VGT TDM	IGTIWEDR	NYFETVPTT	- ALDTALWLESDRMG
	AY IRFILPINGLIVV		VHEDHI			VSTWYHTU	SGDEP - AGK			SUSENNKU	SFFAPLEK-	- VGT TDM	GIIWEDR	NYFEIVPII	
	PERKIKLANGLIVI		LHQDN	S-DPLVH-			SAREQ-LGK	SGFAF							
			LHPDH:	S-DPLVH-			AREE-PGK	SOFAF							
			VIEVKI	S-DPLVH-	UVULATNIL		SAREE-IGR				ODEELMIE	DOLINI TEM		TAVELATONKK	
				I KVTGAV			ERTOP MOS				RDFFFMIR	ROLNTEM		TAVPEATONKK	
			VSEYKI	I-KVTGAV	ΗΥΗΙ ΔΤΟΥ		FRTOP - MDS	KGT			RDPEELMIR	RSLNTEM		TAYPEATONKK.	- DEONLESVYLDAAE
			VSEYKI	I-KVTGAV	ΗΥΗΓΑΤΟΥ	DENVEL - VA	FRTOP - MDS	KGTAF			RDPEELMIR	RSLNTEM		TAYPEATONKK.	
	FKLRSHRISTINVT		VEEYRI	I-RKTGAR		IDENVEE VAI	RTEP - MDS	TGVAL		COSEREEV	RDPFFMMIR	RSINTEM		TAYPEASMNRK.	
	EKIRSHHIGTLNLD		VEEYRI	I- IKTGAR	HLHMAADN	DENVEE - VA	LRTFP - MDS	SGVAL			RDPFFMMIR	RSLN TFM		AYPFASMNRK-	- DFDNLLDVYLDSVF
)FVRSEKIES <mark>L</mark> GID		VAEFTI	H-KETGLT	HYHTAADH	ISENVEL VG	LRTV <mark>P</mark> -EDS	KGV <mark>A</mark> F		_C <mark>GSERFP</mark> V	RDPFFMMTR	RSLN TFM		TSYPFASQNRK-	- DEENLLDVYLDAVE
	3WWRSQVIDSLKIT		VEEYRI	h-kttg <mark>a</mark> a	HYHMAADN	IDENVEL - VAI	LRTV <mark>P</mark> -MDS	TGV <mark>A</mark> ⊢		_C <mark>GSERYP</mark> V	RDPFFMMIR	RSLNTFM		TAYPFASQNRK-	- DFDNLLDVYLDAVF
	JKLRSQRIDS <mark>L</mark> NLT		<mark>L</mark> EEYRI	H - RKTG <mark>A</mark> K	HFHLATD	IPENVEL VAI	FRTV <mark>P</mark> -MDS	TGV <mark>A</mark> ⊢	I I LEHTVI	_C <mark>GSE</mark> KYPV	RDPFFMMLR	RSLNTFM	IAFTSSDW	TAYPFASKNKK-	- DFNNL <mark>L</mark> GVYL <mark>D</mark> AVF
)RIRSQPIDS <mark>L</mark> NLT		VEEYRI	H - RKTG <mark>A</mark> K	HFHLATDN	IPENVEL VAI	FPTV <mark>P</mark> - TDS	TGV <mark>A</mark> ⊢	ILEHT <mark>VI</mark>	_C <mark>GS</mark> RNYPV	RDPFFMMLF	RSLN TFM	IAFTSADW	TAYPFASKNKK-	- DFSNL <mark>L</mark> KIYL <mark>D</mark> AAF
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Figure S5A. Segment of the sequence alignment of the N-terminal of PqqF (M16A peptidases) from γ -proteobacteria. Highlighted in red is the zincbinding consensus HxxEH. Sequences were aligned using Clustal Omega and the figure was generated and analyzed in Jalview (3).

RY region

	1140	1150	1160	1170	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270
		- VHEFTT - AG	GDDA <mark>AVLLFC</mark> F	LAENTA-ECI		LASF-LEPKFFC	QL RVEKNVGY	VSCRFHQT.	AR - QSGI	LFALOSPT	YSAET		LIGFIHTFI	AQIPAI - IDG- I
	PL - PAPI	HRFTLPAN-(GTDS <mark>ALL</mark> LFVF	LVASQR-EA	VT <mark>AL</mark>	HAP <mark>F</mark> FC	NH <mark>RVENNIGY</mark>		AG YE <mark>G</mark> L	LALLQSPHI	LSPDA		LUNETTAF	ERQGET-WRA-V
	PL - PAP	HRFTLPAN-(GTDS <mark>ALL</mark> LFVF	LAASQR-EA	VT <mark>AL</mark>	HAP <mark>F</mark> FC	QHNIRVEKN I GY		AG YE <mark>G</mark> L	LALLQSPHI	LSPDA		LHETTAF	EGQSEA-WRA-V
	PL-PAP	HRFTLPAN-(GADS <mark>ALL</mark> LFVF	[•] LVASQR-EA/	AT <mark>AL</mark>	QAP <mark>F</mark> FC	QH <mark>NR</mark> VANN I GY		AG YE <mark>G</mark> L	LALLQSPHI	LSPDA		LHETTVF	ERQGDA - WRA - V
	P I - AAA	QPVRLSDDR/	ADDRTLL LFYF	<mark>'LA</mark> DATP - TAF	RL <mark>ALRL</mark>	L <mark>A</mark> QL YAPR <mark>F</mark> FC	QRL <mark>R</mark> VELNIGY	AHCAYHRC	· AD - - RD <mark>G</mark> M	ILFAL <mark>QSP</mark> R	CSLEQ		LHNYTREFL	.QQMRQE - LAA - L
	I R - TPA	ERVALAES-(gden <mark>arl</mark> vf yf	PLPETSA - AAF	RW <mark>ALQL</mark>	LAQL YASRYFC	QR <mark>L R</mark> VDQN I GY	AHCAYYRC	VD VE <mark>G</mark> I	FFALQSPK	YTVAQ		MDHYTAAFL	.QQMKLE - LAQ - I
	PR LHI	PPVTLTES-(GAEH <mark>ALL</mark> QF YF	PLQSDEA-EGP	RWALRV	LARL YAPRYFO	RURVERNVGY	V QCTFHRC-	TH VE <mark>GL</mark>	LFALQSPTI	FTAEQ			QQMHHE - LTH - V
	AP - LHH	HPVTLTES-(GAEHALLQFYF	PLPNDEA-EGP	RWALRV	LARL YAPRYFC	QURVERNVGY	V QCAFHRS -	AD AE <mark>G</mark> L	LFALQSPTI	FAVAQ		LRQLTDEFL	LQMRHE - LAH - L
	TH - HHHI	RPVTLTES-(GAESALLQFYF	LPNGEA-EGP	RWALRV	LAQL YAPRYFC	ARL RVDRNVGY	VQCAFHHC	ADAEGL	LFALQSPTI	FNVEQ		LRQLTDEFL	LQMRHE - LAH - V
	IY-QHHI	RPVILIES-0	GAEHALLQFYF	LPNGEA-EGH	WALRV	LAQL YAPRYFC			ADAEGL	LEALQSPT	FNVEQ			LQMRHE - LAH - V
	AA-GGEI	RELHLGI-AU	GSEQAVELRE	APPGEP - RRE	= AAWRL	LATE - WPGAFHO			EE GAEL	QLGVQSPH	VPAAR			VVQQLR-LAG-L
	AL-GGE	HALHLUT - AU	GSERAVLLRL	APVGUP-VVL					GDGAEL	QFGVQSPH/	ASVER			
	SIA(ANU	GARGARI-GU	GSEPALLEC					AVESAEROV	EG VCC		АЗНАQ ТСОЛО		VEDULIEL	
	TIAL ASU	RVIQHAQV-FU Diamuaev/ di	BSENALLE CO					AVESTEDOV			TOQAQ		IL DULL TL	
	IP-GRAI	NWSOVTC-G9	SDEHALL WACE			LAOL - LOVPEY		VESAEROL.		LEGVOSEG	SDCAA			
	TE-PGK	RMATEAS-S	SSEDAVL VECE			LAHV SOAL FYO		VESGIROL	NGRTGI	LEGVOSES			LEOHIETEI	GRIPER-VRD-A
	TE-PGK	RWATEAS - SS	SSEDAVLVEC	APTASI-EDE		LAHV SQALFYC		VESGIRQI	NGRTGL	LEGVQSPS	CDAGQ		LFQHIETFI	GRLPER-VRD-A
	TF - PGK	RWATEAS-S	SSED <mark>AVLVFC</mark> F	ALTASI-EDE	E <mark>AAWRL</mark>	LAHV SQALFYC		AVFSGIRQI-	NGRT <mark>G</mark> L	LEGVQSPS	CDAGQ			GRLPER - VRD - A
	LD - GRKI	RWHAVST-E	SSEA <mark>ALL</mark> LFCF	TPTQSL - ADE	E <mark>ahwrl</mark>	LGHVLQGLFYC	RI <mark>RVELQIGY</mark>	A <mark>√</mark> FSGIRHI-	NGQT <mark>G</mark> L	LFGVQSPS/	ASLDG		IVQQLQAFL	GQLPSL - IER - S
	LE-GQR	IWHEVNT - AS	SSDA <mark>ALL</mark> LFCF	VPSQSL-VDE	E <mark>anwrl</mark>	LGHM LQGP <mark>F</mark> YC	RI <mark>RVELQIGY</mark>		DG QT <mark>GL</mark>	LFGV <mark>QSP</mark> SI	LSLGR		TAEHLQT <mark>FL</mark>	QQLPSL - IKR - S
	PR-GQR	IWNTLKT-HO	GEEP <mark>AVL</mark> LFC	TP TP AL - ADE	E <mark>AAWR</mark> W	L <mark>A</mark> QL CQTL <mark>F</mark> YC	DRI <mark>R</mark> VELQLGY	/VFSGLKQI -	DG QT <mark>G</mark> M	ILFGV <mark>QSP</mark> NI	LSATQ		LSAHIEQFL	AGLPAL - VQQ - L
	AG - TGQ	Q/VQNLPG-S/	AGEH <mark>ALL</mark> LFY <mark>F</mark>	VPSASL-ADE	E <mark>AAWRL</mark>	LGQL CQTPFYC	QRL RVELQLGY	VFSAVRQR-	NGRT <mark>G</mark> L	LFGV <mark>QSP</mark> G/	ATVTE		ILQHIAQ <mark>F</mark> L	EHLPEQ-LQA-L
	NP-APY	RWCRFGD-P(GTET <mark>ALV</mark> LFC	PLPARTP - AVE	E <mark>AAWR</mark> Q	L <mark>a</mark> rl Mega <mark>f f</mark> f	RRI <mark>R</mark> SELQLGY.	FCGFRQF	GG QG <mark>G</mark> L	VFAVQSPS/	ATAGE		LLGHIEAFL	ESFAGQ-LDD-Q
	GR-H	- RREL - A - V[DGES <mark>ALL</mark> LFCF	<mark>PLP</mark> TQEV-PMB	E <mark>AAWRL</mark>	<mark>La</mark> rl Hepa <mark>f</mark> qf	RRI <mark>R</mark> DELQLGY.	LFCGFREV	GA RR <mark>G</mark> L	LFAA <mark>QSP</mark> R/	ACPAR		LEHMETFL	.QRSAEA - <mark>L</mark> AQ - L
	SN-HEL	LWREVAQ-P	GS <mark>DA<mark>AL</mark>VLLC<mark>E</mark></mark>	LADDEP - RSE	E <mark>AA</mark> WRL	<mark>L</mark> GQL - - LQGA <mark>F</mark> YF	RRIRGELQL <mark>GY</mark>	ALFAGYRQV ·	QG ERA <mark>L</mark>	LFALQSPV0	CEAAG		IFAHIRS <mark>F</mark> L	DQQCQQ-IAE-L
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	DG++QPL PPPAPQ	GM TLPTSA	GSD+ALLLECE	PLP+ASA+PDI		LAQLR+LQPREEC	RI RVEQQLGY	VVSCR+Q+V	AD+RRDGL	LEALQSPS'	YS+GR+++IEC	WIRPLRSNNE		ROL + +L LL AAGL

Figure S5B. Segment of the sequence alignment of the C-terminal of PqqF (M16A peptidases) from γ -proteobacteria. Highlighted in red is the RY region characteristic of the C-terminal of this type of M16 peptidases. Sequences were aligned using Clustal Omega and the figure was generated and analyzed in Jalview (3).

	G-rich R-Y	' region
90 300 310 320 330 34	0 350 360	370 380 390 400 410 420 430 440 450 460 470 48
PAAATFGHGGSRVVHRDLEQAHLTLALEGVPQTDGSLFSLQ	FTSVLGG-GM: SRLFQEVREKRGLCY	S IÝTFHAÞYADTĠLEGLÝTGTDPSDAPÉMMEVÍVDVIŠEAV-ÉTLTEÁE IARÁKAQMKAGLLMA-LEŠCSARÁEOLARHMLVÝGRP-ÓSVGELTARIDAVSVÉSTRDÁARGLÍV
PEKAALELTGGVTMIDNDSPQTVVSFAQPGLPMSDPDYFAAY	ADHILGGGGFSSRLMDEIREKRGLT	CVRTGLANGVYGETWQGGMASANDKVAEAVGLIRSEWDRFAEGOVTEKELADAKTYLTGEYALR-FDGNGKIAGILAGMQ-LIGLPADYVNTRNGKIEAVTAEDVQRVAQRLL
		UTGSYLEPLDDAALYTGGESSDNIRTAEATAY THEOWADTAARGYTAEELEAN YYTGGYPEN-HOGNGRTASTLEVGMO-ODDLSTDTTPTRNDKVRAVILEDTARVAARLI I GSYLEPLDDAALYTGGESSDNIRTAEATAY OD VDEDAADTAARGYTAEELEAN YYTGGYPEN-HOGNGRTASTLEVGMO-NGCTDTPTRNDKVEAVILEDTAVAAL
PEDVTVETEAGVTV/DEPTPOSVALEGHRGMKRNDPDEEAAYN		
PPDVAAETTAGTTVVPFDTPQSVAVFGHEGLAIDDPDFFAAY	MNTILGGGSFESRLMNEVREKRGLT	QYYSYLAGMDHAELLMGRVASANDRIAEAISVIRDEWAKMASEBYTEQELEQTKTYLTGAYPLR-FDGNGTIARILVGMQ-MDGLPADYIASRNDRIEAVTRDDVKRVAARVLF
PADIEAETSAGVTVVPFDTPQSVAMFGHAGIARDDPDFFAAYV	LNQILGGGGFEARLMTEVREKRGLT	QVYSYLVPMDHSALYLGRVASANDRIAEAISVIRDEWRKMAEDGVTAAELEQAKTYLTGAYPLR-FDGNGPIARILVGMQ-MDGLSPEYVTRNAEVEA <mark>VT</mark> RDDVARVAARLLI
PPRANVEISGGITVVPFETPQSVARFAQKGIKLDHPDYFTAVV	/LNHV_GGGSFESRLMDEVRAKRGLT	CVYSYLAGKDLAEVYIGSVSSANDRIAEAIEVIRDEWEKAATEGVTAGEVENANTFLTGAVPLR-FDGNOPIANIMVGMO-MLGLPIDVIATRNERVEAVTVEGVKRVAGELL
		UVYSYLAGRDLAEVYIGSVSSANDRIAEAIEVIDEWEKAAIEGVIAE VENAKIFLIGAYPLA-FUGNOPIANIMVGMO-MLGEPIDVIAIRREGIAAVIVGKVKRVAGELE VVYSYLVDDIAAVVGSVSSANDRIAEAIEVIDEMEKDADAENDVIEVELKIKAALEDVIAEALEDIAEUKAALEDI
PERAQVSVPGPVRVVDEDTPQSVALEVOPGIDRDDPDEETAYI		
VVDAVP-KLGRAIRVAYDLPQAQLSLAYPGIPRKDPQFFAANL	MNQILGGGAFTSRLWNEVRE (RGLA)	CIYSTLENIDHASALVIGTGTRPDRAAETLSLIQAEVRRMSEEBVSEDELTAAKKKLIGGYAIENLNSSSAVAQTLVQIQ-LEDRGIEYVERKQLIQAVTVEDVRAVAKRLLS
VADIQP-EFGERVAIDLAVPQTTIQFALPGVKRDDPEFFAAYL	MNHV_GGGSFTSRLYEEIREKRGLAN	GASSWLASYEHAAILGASTATRADAAEESIRIIREELERMAEE <mark>GPTEDEL</mark> AMAKTYYKGSYAYRNL <mark>D</mark> SSGAIARTLYGIQ-LDDLGMD <mark>Y</mark> IDTRODQIDA <mark>YT</mark> MDQYKAAAQKLLS
VAKTE-PTGGSQRVIEMGVPQSVAIFGLGAMPRKDPDFMAAFI	IINHILGGGGFSAKLMEEVREKRGLA	SVYSYVQPDKITSILVGSVATKNASMSESLDIIRNEMKKMAENOPTEADLDAAKSYLTGSYALR-FDTNSKIASQLLGLM-QEGFGPDYVENRNKMIDAVTLADAKRVAARLLF
		GYFESLUWIERSAYFIGYIGTRADRAGDIIDAIDREVRFIAEEDFICKEUDEANSTURGSONLA-LDISSKLAALLGIG-GUDREFIDTERRYAIVDAYTLDDAKAAARRUW GYFESLUWIERSAIFIGYIGTRADRAGDIIDAIDREVRFIAEEDFICKEIDEANSYLKGSONLA-LDISSKLAALLGIG-GUDREFIDTERRYAIVDAYTLDDAKAAARRUW
		YEOLUWICHSALF (STGTRADRATET IDAITAEVKRIGECOPSECELAEAKSYINGSOMLS-LDTSAKLAQALLOYO-NDGLPIDYIDKRSEVVNAVTLADAKRAAORLW
VPHVEPQKIGTVDVIPLDVPQSVVVMGTGGLERRDPDFIPAFV	LNHILGGSAFSSRLFKEVREARGLA	SVYSYQVALGHTGLWFAGTATKNERAGESIAIITDEFRKILKD <mark>G</mark> PSQT <mark>EL</mark> DEA <mark>K</mark> SYLMGSYALR-FDTSSKVAGQLLQIQ-LDELGIDYVDRNALIAAVTLDDLKHVAARLA
VPPTRITGLGRRVVVDLDVPQSVIRFGTDGVPWRDPDFIPAYV	LNHILGGGAFTSRLFQEVREKRGLA	SYGTSLVSHRAAS I TWGSTATKNERVGEALSV I GEE I ARLTRD <mark>G</mark> PSDDELQKAKDYLTGSYALG - FDTSTK I AHOLVQVA - FEGLG I D <mark>Y</mark> I GRRNGL I AA <mark>VT</mark> QED I RRAARRTL(
VPPTR I AGLGTAGSGRR I VVDLDVPQSV I RFGADGVPWRDPDF I PAYV	LNHI_GGGAFTSRLFQEVREKRGLA	GYGTSLVSHRAASMWGYTATKNERVAEALSVIGEE IARLTROGPSDEELOKAKDYLTGSYALG - FDTSTKIAHOLVOVA - FEGLGIDY ISRRNGLIAAVTODDIRRAAARTL(
		SVGISLVSHRAASMINGYIAINNERVGEALSVIGEEIANLINDEPSDELGNAMUNLIGSYALS-FGISIEIAGNLVUVA-FEGLGIDIISKRNGLIAANGUDIIKAAARTU GVGTSLVSHRAASMINGYIAINNERVGEALSVIGEEIGIIAAGENIDAUGNAMUNLIGSYALS-FGISIEIAGNLVUVA-FEGLGIDIISKRNGLIAANGUDIIRAAARTU
VPPTAINELGRRIVVDLDVPQSVIREGMPGVAWRDPDEIPAY		
IPPTAINEL GRR IVVDLDVPQSVIRFGMPGVAWRDPDFIPAY	LNHILGGGAFTSRLFQEVRE RGLA	SVGTSLTSHRAVAMTWGYTATKNERVVEALDV I GDE I ORL I TOBPSDEEL OKAKDYLTGSYALG - FDTSTK I ANOLVQI A - FEGLGMDY I ARRNDLVASVTQAD I RRAGARTL(
IPPTAINELGRRIVVDLDVPQSVIRFGMPGVAWRDPDFIPAYV	LNHI_GGGAFTSRLFQEVREKRGLA	SVGTSLTSHRAVAMTWGYTATKNERVVEALDVIGDEIORLITD <mark>G</mark> PSDEELOKAKDYLTGSYALG-FDTSTKIANOLVQIA-FEGLGMD <mark>Y</mark> IARRNDLVAS <mark>V</mark> TQADIRRAGARTL(
IPDQLFTGEGRRQVVDLDIPQSTIRFGRQGIGRKDPDFIAATV	/VNHILGGGIFSARLFREVREKRGLA	YYSQLYTFDHGAMLTGGTSTKNERVAESLSVIEEQIRDLSENGPTGEELDKARKYLIGSYALR-FDTSTKIAGQLYHLQ-TDGFDVDYLDARNOWIAAVTMDDAKRVCKRLF(
TPEAEFSSL GQRFVVDVDVDVPQSTIRFGRPGLAQRDPDFFAGM	/VNHVLGGGVFSARLFREVREKRGLA	SVYSOLLNYDHGAMLYGGTSTKNERAAESMAVIEAEIRNLSEVEPTEEELDKAKKYLISSYALR-FDTSTKIASOLLHLO-TDGFDVDOLDERNRFIAAATMEDAKRAAKRAF
		SYTSHENE TUHCAMVYGGAATKNEKARESTUDYTGSGFADEGANDFTADEGUKANKYETGSYALF-FUTSTKTASGEVNEG-TUDGFESTEDERNAKTDAYTMEDARKVANKEU Gyssnespydrager igistrudaatai bytenti oofieersaategaategaatai igastategaatai beskutigyigi yoofi teiddyykevaategoveraadegu
GTDYQELQAAMK GQT LOLDHPAAQSH LLMGLPAMRGAPDYEDLL		
ILPDVKMPLAKAETIRIPHPAQQATIIIGQPGIARGDKDYFPLL	GNYVLGGGGFSARLTNEVRE (RGLT)	SIGSYFAPAAQPGPFELALQTRKDQTEEALGVVRDTVAKFVADGPTDAELKAAKDNLVNGFPLR - LDSNRKLLDNVAN IG-WYNLPLDYLDTWTQR I AA <mark>VT</mark> RDQVRTAFQRVL(
LPAIAPVAALAAPKDERIPFASA <mark>Q</mark> AHVLI <mark>G</mark> QP <mark>G</mark> YPRKDP <mark>D</mark> HFALTL	_GNYV_GGGGFTSRLTNEVREKRGLA	SVYSGFAPGLDAGAFRVGFQTRPDQAEEAVKVSRDVLARFVAE <mark>G</mark> PTAA <mark>EL</mark> KA <mark>AK</mark> DNLIGGFPLL-L <mark>D</mark> SNRKLIGNVANIA-WHDLPLD <mark>Y</mark> LDTWTARMDA <mark>V</mark> TAAEVRAAFARKL(
LPTVPEVEPLAE AQEKIIPFDSAQAHVLIGQPGFKRADPDYFPLTV	/GNYILGGGGFYSRLTSEVREKRGLT)	GISSSFSPGLHAGSFTVGLQTRPDQTAQAVQIARQVVRDFVAG <mark>G</mark> PTEAELKAAKDNLVGGFALR-IDSNRKLLGNLAGIA-WNGLPLD <mark>Y</mark> LDTWTRQVEK <mark>V</mark> TVADVKAAFARKL(
		NYYSYFAAGLHAGAFTVGLIGTRPDGAAEAVGIAREVIAREVADGPTEAELRAAKDOLIGGFALR-IDSNEKLLGNVANIA-WNDLPLDVLGOXSOVQALTVADVRAAMARKL(
		SY 15 FMPC LOGGEFOLGUTINSOMINDALINE VINGVMINDFLENGFT REELKAAMINE IGGEFER-LESINKI AETISMMA-ITTIFFID LESEVINI DIN VEDINDAFURRI. Gyvsyempinteagefolgu otkindaeaau auveetu nikei knowteaetukaakan uggepme. Losinki udvi svug, eyku punni orgynkreventaaduk daenrri.
IPPVAYPTKAIEQRIVHPASQSHILLGYPGIKRGDPDLFPLY		SYYSYFMPMAELGFFQIGLGTKKDOADDALKLYRETDKFLKDEVTESELKAAKANIIG6FPMR-IDSNKKILDYLAVIG-FYKLPLSYLDDYNAKVASYTTAQIKEAFNRRL
LPEVAYPQAAVERRIPHPASQSHIMLGYPGVKRGDPDYFALYV	/GNYI <mark>_GG</mark> GGF <mark>Y</mark> SRLTEEVREKRGLVI	SVYSYFLPMQQLGQFQIGLQTKRDQADDALRLVRETLANFISK <mark>O</mark> VTEA <mark>ELKAAKONIVGGFPL</mark> R-I <mark>D</mark> SNSKILDYLAVIG-FYRLPLN <mark>Y</mark> LDEFNGKVEA <mark>V</mark> TAAQIKDAFSRRI[
L PAVTFPER AVDER I QHPATQSH I LLGYPGVKRGDADYFPL YV	/GNYI_GGGGFTSRLTEEVREKRGLVY	YYSYFMPMAELGPFQIGLQTKREQSAEAMKVVEQTLEKFMQG <mark>G</mark> VTEA <mark>ELVAAK</mark> QNITGGFPLR-L <mark>D</mark> SNSKILDYLAVIG-FYKLPLT <mark>Y</mark> LEDFNKNINS <mark>V</mark> TTAQIKDAFSRRII
	GNYT_GGGGFTSRLTSEVREKRGFA	SVYSYFVPOOVAGIFOIGLOTRSSOAGEALDVVRRTLAGFIAEOPTAAELKSAKONLINGFGLR-LOSNRKILDNVAMIG-FYRLPLDMLDTVPRKVEAVTAEOVRDAFARVI
		SYTS YFAP LKUIGF QIGLOTKKSGARDA I KVARDVLEGF LKOPSDDELAAARANLI ISSFPLK-LDSTIKKTLDINVAVTG-FYGLPUDTDUQVAKVGAVTADE Chinsekpi kvardee tvi otrodoa ika, si tooti kkei toopstel vaakaan al isseples. Isaala si eki e evoi di divatavav
LPPVPDLKRAKIVRRSFDSSQTHILIGAPAISRTNSHYIPLY		
LPPVPMDQP AQTLRRAFPSEQTHVYSGQPGMRINDPDYFPLYV	GNHILGGSGLYSRISEEVRE (RGLS)	SAHSHFYPFRVEGPFLMGLQTRNEKADEALTVLLQTLRDFIAKGPSDKELDAAKKNIVGGFVLR-LDSNOKLVEQIASMA-FYGLPLDYLATYIPKIEAVTKDDVRRWQARII
IAEVQSLTESALVSISYPSSQTTILVGQTGISRDDPDYFPLYV	/GNH I _ GGSGLYSQLSDE I REKRGLT)	QVYSYFRPMCKQGPYQLGLQTRNDQTOEALSVLKQTLNTFIEN <mark>G</mark> PTEO <mark>E</mark> ITAAKONITGGFALR-VDSNNKIADYLSMIG-FYNLPLDYLDSFNDKVKA <mark>V</mark> TVADIKDAFKRRIF
LPSVPSLEEASREVITYPSTQTTVILGTVGMRRGDPDYFPLYV	/GNHV_GGSGLYSRISVELREKRGLTY	SAYSYFSPMRRRGPYILALQTRNEQAEEALQVLRNTLKEFMTRGPGEEELQFAKQNITGGFPLR-IDSNGEKVQYLAMIG-FYRLPLDYLETFTSQVEA <mark>VT</mark> VAQIREAFQKRVI
LSPVPKIKKTEIETIHYPSSQTTIILGTIGVRRGDPDYFPLY	GNHV GGSGLYSRISVELRE (RGLT)	SAYSYFSPMRRRGPYVLSLQTRNEQAKEALEVLRETLQNFIATOPSEKELQLAKONITOGFPLR-IDSNGEKVQYLAMIA-FYQLPRNYLETFISQVEAVTATQIREAFOKRI(
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03242002112322415353*74727+21+7243448786769	17+579**858389+52+9*47+*+6*	8755384432134462736683345627783763346336533+6843+9338+36+5787694-8+7932672285266-5234455+9445433844+8344643664336
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I PAVPPVAAPKI +T+G+GGRTVVD+DVPQSVIREGOPGIKRDDPDEEPAYV	GNHILGGGGEVSRI MOEVREKRGLAN	SVYSYLAPMDHAGPFQGG+ATKNDRAAFAL+VIRDEL+RFA+DGPTEAFL+AAKDYLTGSYPLRNEDSNGKIAGQLVGIQLFYGLPLDYLDTRNAK+FAVT++DIKRAAARLIC

Figure S6. Segment of the sequence alignment of PqqG (M16B peptidase, non-peptidase homolog chain) from α -proteobacteria. Highlighted in red is the RY region characteristic of the C-terminal of this type of M16 peptidases. Sequences were aligned using Clustal Omega and the figure was generated and analyzed in Jalview (3).



Figure S7. Idealized representation of the interaction between the crosslinked PpqA* peptide (green sticks) and the PqqF/PqqG heterodimer protease (blue and pink surfaces). The dimer structure was modeled on the open conformation of *Sphingomonas* sp. A1 dimer (PDB file 3amj, chains C+D) to show the peptide fitting in the active site. (*A*) Front view looking straight at the dimer interface; (*B*) Rotated view (45° degrees about the vertical axis); (*C*) Close-up of the peptide. The gap near the metal center (grey with coordinating residues in orange) and R/Y pair (magenta) suggest a possible ingress path for the peptide. Because the heterodimer is represented in the open clam shell conformation it is unlikely that the R/Y pair is interacting with PqqA* in this situation. Also note that the cross-link between residues Glu15 and Tyr19 of PqqA*, in red, may help in stabilizing a helical conformation for the peptide.

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