

Supplementary material

Table S1. Sequences of primers used.

Primer name	Sequence
<i>Eg_GSP_PCS_Fw</i>	5'CATGTAGGTTGCGTCACACGGAT3'
<i>EgPCS_ORF_Fw</i>	5'CGTACATATGGCAACGATGTGGGC3'
<i>EgPCS_ORF_Rv</i>	5'TAGCGGATCCTCAGCCGCTGGCGCAG3'
<i>EgPCS_pE_Fw</i>	5'CACCATGGCAACGATGTGGGCCGCGGCCA3'
<i>EgPCS_pE_Rv</i>	5'GCCGCTGGCGCAGACGCCTGTCGTC3'
<i>EgPCS_Nter_USER_Fw</i>	5'GGGAAAGUATGGCAACGATGTGGGCCGCGGCC3'
<i>EgPCS_Nter_USER_Rv</i>	5'GGAGACAUGGCGGAGAGCAGCACGTAC3'
<i>EgPCS_qPCR_Fw</i>	5'GAGCAGTTCCAGAAGCATGTC3
<i>EgPCS_qPCR_Rv</i>	5'TCGAGCGTGAATACGACACTA3'
<i>EgAct_qPCR_Fw</i>	5'AAAGGAAGTACAGCGTGTGGA3'
<i>EgAct_qPCR_Rv</i>	5'CCTCGTCGTACTCCTCCTTCT3'

Table S2. NCBI *Gene Identification* numbers of the phytochelatin syntheses protein sequences analyzed.

Taxa	GI number
Viridplants	
<i>Arabidopsis thaliana PCS1</i>	75198390
<i>Leucaena leucocephala</i>	215983522
<i>Thellungiella halophila</i>	312283437
<i>Amaranthus tricolor</i>	63413051
<i>Brassica juncea</i>	18958247
<i>Thlaspi caerulescens</i>	21104518
<i>Thlaspi japonicum</i>	21104516
<i>Noccaea caerulescens</i>	220683835
<i>Arabidopsis thaliana PCS2</i>	15219610
<i>Arabidopsis halleri PCS2</i>	325516458
<i>Arabidopsis lyrataPCS2</i>	297848618
<i>Noccaea caerulescens</i>	166798217
<i>Nicotiana tabacum</i>	29470177
<i>Sesbania rostrata</i>	67906844
<i>Glycine max homoPCS</i>	351725998
<i>Pyrus betulifolia</i>	373405317
<i>Lactuca sativa</i>	53760453
<i>Sonchus arvensis</i>	255964729
<i>Triticum aestivum</i>	5757804
<i>Hordeum vulgare</i>	326494126
<i>Sorghum bicolor</i>	42051649
<i>Cynodon dactylon</i>	33187146
<i>Oryza sativa</i>	115465870
<i>Populus trichocarpa</i>	224129604
<i>Glycine max</i>	356505041
<i>Lotus japonicus</i>	388497750
<i>Fagopyrum esculentum</i>	156138741
<i>Selaginella moellendorffii</i>	302782732
<i>Chlamydomonas reinhardtii</i>	159485978
<i>Volvox carteri 1</i>	302847090
<i>Chlorella variabilis</i>	136723
<i>Coccomyxa subellipsoidea C-169</i>	384245625
<i>Volvox carteri 2</i>	302843401
<i>Athyrium yokoscense</i>	15617416
<i>Pteris vittata</i>	313483731
Opisthokonts	
<i>Eisenia fetida</i>	148807788
<i>Schizosaccharomyces pombe</i>	19114464
<i>Schizosaccharomyces japonicus</i>	213409680
<i>Wallemia sebi CBS 633.66</i>	388578797
<i>Crassostrea gigas</i>	405962103
<i>Hydra magnipapillata</i>	449668511
<i>Caenorhabditis elegans</i>	14485518
<i>Caenorhabditis brenneri</i>	341888907

<i>Brugia malayi</i>	170594708
<i>Ciona intestinales</i>	198417053
<i>Schistosoma mansoni</i>	256052410
<i>Tuber melanosporum</i>	296417443
Stramenopiles	
<i>Aureococcus anophagefferens</i>	323451775
<i>Phytophthora infestans</i> T30-4	301112447
<i>Phytophthora sojae</i>	348672680
<i>Thalassiosira oceanica</i>	397645345
Amoebozoa	
<i>Acanthamoeba castellanii</i>	470517486
<i>Dictyostelium discoideum</i> AX4	66803020
<i>Dictyostelium purpureum</i>	330794008
<i>Polysphondylium pallidum</i>	281202856
Haptophye	
<i>Emiliania huxleyi</i> CCMP1516	485640797
Exacavates	
<i>Euglena gracilis</i>	This work
<i>Naegleria gruberi</i>	290980155
Rhodophyte	
<i>Cyanidoschyzon merolae</i>	CMI111C*
Cyanobacteria	
<i>Nostoc</i> sp. PCC 7120	17130321
<i>Anabaena variabilis</i>	75702893
<i>Nostoc punctiforme</i>	186465411
<i>Chamaesiphon minutus</i> PCC 6605	434384310
<i>Cyanothece</i> sp. PCC7822	307153464
<i>Synechococcus</i> sp. PCC6312	427714658
<i>Crinalium epipsammum</i> PCC 9333	428304353
<i>Crinalium epipsammum</i> PCC 9333	428304353
<i>Microcoleus vaginatus</i> FGP-2	334118092
<i>Oscillatoria nigroviridis</i> PCC 7112	428317750
<i>Coleofasciculus chthonoplastes</i> PCC 7420	254413464
<i>Coleofasciculus chthonoplastes</i>	493032501
<i>Geitlerinema</i> sp. PCC7407	428225164
Proteobacteria	
<i>Burkholderia thailandensis</i>	492895672
<i>Burkholderia mallei</i>	490309833
<i>Coxiella burnetii</i>	207082025
<i>Polynucleobacter necessarius</i>	145588734
<i>Rhodobacteraceae</i> bacterium HTCC2083	497514158

* Sequence retrieved from <http://merolae.biol.s.u-tokyo.ac.jp>

Table S3. Non-contiguous amino acids (153) from the PCSases Nter protein sequences used in the phylogenetic analysis.

PCS	aa
	1121314151
ACC81212_Nostoc_punctiforme	FKVQLPIPFASPEGKRVFREAGGLGNFFFEVAEQFHTQNDPAYCGPGIVMALNSLLDPQRM
Q9S7Z3_Arabidopsis_thaliana	QTLPLSIGFNTPEGEKLLFESKSNQDFFSLSMQFTTQNNQAYCGVAMVMVLNGLIPPEAS
ACJ71777_Leucaena_leucocephala	YRRSLPIDFSSAEGKLI FNEAGTMEGFFRLISYFQTQSEPAYCGLALSVVLNALIDPGRK
BAJ34584_Thellungiella_halophila	YRRSLPIDFSSAEGKLI FNEAGTMEGFFRLISYFQTQSEPAYCGLALSVVLNALIDPGRK
AEW23125_Amaranthus_tricolor	YRRSLPIDFSSAEGKKI FNEAGTMEGFFRLISYFQTQSEPAYCGLALSVVLNALIDPGRK
BAB85602_Brassica_juncea	YRRSLPIDFSSAEGKKI FNEAGTMEGFFRLISYFQTQSEPAYCGLALSVVLNALIDPGRK
BAB93120_Thlaspi_caerulescens	YRRSLPIDFSSAEGKLI FNEAGTMEGFFRLISYFQTQSEPAFCGLALSVVLNALIDPGRK
BAB93119_Thlaspi_japonicum	YRRSLPIDFSSAEGKLI FNEAGTMEGFFRLISYFQTQSEPAFCGLALSVVLNALIDPGRK
ACL80669_Noccaea_caerulescens	YRRSLPIDFSSAEGKLI FNEAGTMEGFFRLISYFQTQSEPAFCGLALSVVLNALIDPGRK
NP_171894_Arabidopsis_thaliana_PCS2	YRRSL-IDFASFEQGKIFNEAGTMEGFFRLISYFQTQSEPAFCGLALSMVLNSLIDPGRK
ADZ24787_Arabidopsis_halleri_PCS2	YRRSLTIDFTSVEGKQIFNEAGTMEGFFRLISNFTQSEPAFCGLALSMVLNSLIDPGRK
XP_002892190_Arabidopsis_lyrata_PCS2	YRRSLTIDFASVEGKQIFNEAGTMEGFFRLISYFQTQSEPAFCGLALSMVLNSLIDPGRK
ABY89660_Noccaea_caerulescens	YRRFLPIDFASVEGKQIFHEAGTIEGFFRVISYFQTQSEPAFCGLALSMVLNSLIDPGRK
AAO74500_Nicotiana_tabacum	YRRVLPVDFASTEGKQLFLEAGTMEGFFKLISYFQTQSEPAFCGLALSMVLNALIDPGRK
AAAY82881_Sesbania_rostrata	YRRLLPVDFASSQGKQLFLESGTMEGFYRLVSYFQTQSEPAFCGLALSMVLNALIDPGRK
NP_001235576_Glycine_max_homoPCS	YRRLLPVEFASQGKQLFLESGTMEGFYKLVSYFQTQSEPAFCGLALSMVLNALIDPGRK
AEY68568_Pyrus_betulifolia	YRRILPVEFASLQGGKLFLEAGTMENFYRLISYFQTQSEPAYCGLALSMVLNALIDPGRK
AAU93349_Lactuca_sativa	YRRVLPIDFASIEGKQLFLEAGTMEGFFKLISYFQTQSEPAYCGLALSMVLNALIDPGRK
ACU44656_Sonchus_arvensis	YRRALPIDFASSEGGKLFMEAGTMEGFFKLISYFQTQSEPAYCGLALAMVLNALIDPGRK
AAD50592_Triticum_aestivum	YRRVLPVEFASAEGRKLFMEAGTMEGFNLI SYFQTQSEPAFCGLALSVVLNALIDPGRK
BAJ85525_Hordeum_vulgare	YRRVLPVEFASAEGRKLFMEAGTMEGFNLI SYFQTQSEPAFCGLALSVVLNALIDPGRK
XP_002454970_Sorghum_bicolor	YRRVLPVDFASPEGKRLFMEAGTMEGFFPLVSYFQTQSEPAFCGLALAVVLNALIDPGRK
AAO13810_Cynodon_dactylon	YRRVLPVDFNSPEGKRLFSEAGTMEGFFSLISCFQTQSEPAFCGLALAAVLNALIDPVRK
NP_001056534_Oryza_sativa	YRRVLPVEFASAEGRKLFSEAGTLQGFFNLI SYFQTQSEPAFCGLALSVVLNALIDPGRK
XP_002320627_Populus_trichocarpa	YRRALPIEFASPEGKQLFTEAGTMNSFFKLISYQTQSEPAYCGLALAMVLNALIDPGRK
XP_00352130_Glycine_max	YRRVLPIDFASPEGKLFGEAGTMQGFKLISYQTQSEPAYCGLALSVVLNALIDPGRK
AFK36941_Lotus_japonicus	YRRVLPIDFASPEGKKIFVEAGTMEGFFKLVSYYQTQSEPAYCGLALTIVVLNALIDPGRK
BAF75863_Fagopyrum_esculentum	YRRNLPIDFASLEGKLLFVEARNMEGFFKLISYQTQSEPAYCGLALSMVLNALIDPGRK
XP_002973139_Selaginella_moellendorffii	YRRVLPIDFTCREGKEIFREAGTMEGFFKLIAHFQTQAEPAFCGLALSMVLNSLIDPGRK
105477_SelaginellaUGI	YRRVLPIDFTCREGKEIFREAGTMEGFFKLIAHFQTQAEPAFCGLALSMVLNSLIDPGRK
A816U6_Chlamydomonas_reinhardtii	-----GRQLFQAEAGTMTGFFKLMEQFNTQDEPAFCGLALAMTINALIDPRRT
302847090_Volvox_carteri	YRKRLPIEFSSPEGRQLFQAEAGSMVGFYKLMQFNTQDEPAFCGLALAMVLNALIDPRRT
136723_Chlorella_variabilis	YRKQLPIEFASARGKQVGEAGTVNNFFKLIEQFRTQDEPAYCGLALAMTINTLIDPRRT
384245625_Coccomyxa_subellipsoidea	-----KQGLFAEAGTMEGFFKLIEQFRTQDEPAYCGLALAMVINTLIDPRRA
302843401_Volvox_carteri	YKRVLPISFSSAEGRQIFSEAGTMVGFYKLMQFNTQDEPAYCGLALAMTINALIDPRRT
BAB64932_Athyrium_yokoscense	YRRVLPIDFASQEGKVLFAEAGTMNGFFRLISYFQTQAEPAFCGLSLTVVLNALIDPGRK
ADR51694_Pteris_vittata	-----MNGFFKLVSFTQTQAEPAFCGLSLTVVLNALIDPGRK
428304353_Crinalium_epipsammumPCC9333	YRRALPIAFSSAQGRKIFAEAGGMEGYFALAEQFHTQVEPAFCGLGLVVVLNALIDPGRK
428304353_Crinalium_epipsammum_2PCC9333	YRRALPIAFSSAQGRKIFAEAGGMEGYFALAEQFHTQVEPAFCGLGLVVVLNALIDPGRK
485640797_Emiliana_huxleyiCCMP1516	HRRVLPIDFASSTRGRLEFAEAGTLHCFFKLIEQFCTQAEPAFCGLGLVVVLNALIDPGRK
ZP_08492182_Microcoleus_vaginatus	YRRSLPIEFSSVEGRQIFREAGGMEGYFALAEQFHTQAEPAFCGLGLVVVLNALIDPGRK
'428317750_Oscillatoria_PCC_7112'	YRRSLPIAFSSVEGKQIFREAGGMEGYFALAEQFHTQAEPAFCGLGLVVVLNALIDPGRK
ZP_05027234_Microcoleus_chthonoplastes	YRRPLPIFVSSSTEGRQIFREAGGMEGYFALAEQFHTQAEPAFCGLGMVVVLNALIDPGRK
493032501_Coleofasciculus_chthonoplastes	YRRPLPIFVSSSTEGRQIFREAGGMEGYFALAEQFHTQAEPAFCGLGMVVVLNALIDPGRK
428225164_Geitlerinema_PCC7407	YRRPLPIAFSSSTEGRTIFREAGGMEGYFVLAEQFHTQADPAFCGLGMVVVLNALIDPGRK
470517486_Acanthamoeba_castellanii	FRRELPIFSSSDEGKKLFREAGFMENYFTLAAQYRTQSEPAFCGLGLTMAINALIDPGRV
XP_635353_Dictyostelium_discoideum	YKRNLPVEFSSDEGKKLFRESGHMEGYFSLAEQFVSQSDPAFCGLALAMVLNALIDPNRL
330794008_Dictyostelium_purpureum	YKRDLPIDFSSSEEGKLI FKEAGNMENYFSLAEQFQLSQSDPAFCGLALAMVLNALIDPNRL
EFA77058_Polysphondylium_pallidum	YKLSLPIDFSSSEEGKKIFREAKYMEGFVFLAEHSTPQSDLTNCSLALAMVLNSLIDPKRI
323451775_Aureococcus_anophagefferens	YKRLPFAVDFSKGRAHFESAGGLNFVFLAQFQTQNEPAFCGLALAMINALIDPGRV
ABR13683_Eisenia_fetida	YRRPLPTAFCSPEGKSI FREAGYMENFFFLASQFRTQEEPAFCGLSLVMVLNTLIDPGKV
NP_593552_Schizosaccharomyces_pombe	YKRLPLAFDSSSLGKDVFLRAGRMENYFSLAQQMVTQNEPAFCGLGLCMILNSLIDPGRK
XP_002175610_Schizosaccharomyces_japonicus	YKRLPLAFDSTEGKRLFQAGGMENYFSLAQFVTQNEPAFCGLGLCMILNSLIDPGRK
EIM19133_Walleimia_sebi	YKRLPLTSFTSSRGKEMFANAGNMEAYFPLAQFITQNEPAYCGLGLVMIINALQDPGRK
EKC27807_Crassostrea_gigas	YRRVLPISFYSEEKGI FKEAGHMECYFKLAAQFRTQDEPAFCGLTLVIALNALIDPGAV
XP_002156317_Hydra_magnipapillata	YKRLPLAFSSSEEGKQVKEGSGYANIFPLAQFRTQAEPAFCGILSLVMILNSLIDPKRV
AAK62991_Caenorhabditis_elegans	YRRPLPIEFSSSELGKKLFTEAGSANIFYKLASQFRTQDEPAYCGLSLVMVLNALIDPEKV
EGT44842_Caenorhabditis_brenneri	YRRPLPIEFSSIEGKRLFTEAGSANIFYKLASQFRTQDEPAYCGLSLVMVLNALIDPEKV
XP_001902100_Brugia_malayii	YKRLPLINFASSEGGKLFIESGHANIFRLASHFLTQDEPSYCGLSLVMVLNALIDPGRV
XP_002697994_Phytophthora_infestans	YQRELPAVAFSSLEGRKLFQSQGNTHSFFPLIEQFHTQAEPSYCGLSLVMVNMNALIDPKRV
348672680_Phytophthora_sojae	HRRHLPIDFSSPEGRKLFTEANYMQIFYFLAEQFITQAEPAFCGLALAMCNALIDPGRK
XP_002128372_Ciona_intestinales	HRRHLPIDFSSPEGRKLFTEANYMQIFYFLAEQFITQAEPAFCGLALAMCNALIDPGRK
EJK76788_Thalassiosira_oceanica	YRRQLPIFSSSDDGKLI FKEAGGMEIFHFLGPQIRTQDEPAYCGLSLVIVLNLIDPGRV
XP_002569764_Schistosoma_mansoni	YRRQLPIFSSSAEGKRLFASAGGAHTFFFLIEQLQTPPEPAFCGLTLVVVLNALIDPGRS
CM1111c_Cyanidoschyzon_merolae	YRRPLPIFSSSPEGKHI FKEAGHMEYSYFLASQFCTQEEPSYCGGLALVMIINALIDPGRV
XP_002838367_Tuber_melanosporum	YRRPLPIALDSAEGRALFERSGLAEFFFLVLSQFTTQSEPAFCGLGLAMVLNALIDPGRK
BAB72932_1_Nostoc	YMRLEPIGYDTPGKRLFAPAGGLEAFFFLSQQFLT---VYCGIGLCMIINALIDPPTVT
ABA22569_Anabaena_variabilis	QTLTSLIGFNSNEGEKLLLTLSRSREDFFLPSMQFVTQVNVQAYCGVAIIMVNLINLPETA
434384310_Chamaesiphon_minutusPCC6605	QTLTSLIGFNSNEGEKLLLTLSGSREDFFLPSMQFVTQVNVQAYCGVAIIMVNLINLPETA
307153464_Cyanothoe_PCC7822	QTLPLPIFSSSDGEQLLVSQAKQDYFLLSNQFVTQINQAYCGVASVMVLNALLPPESA
492895672_Burkholderia_thailandensis	QTLPLAINFNSTEGEKL LLSQKARQDCEPFLSIHFLTQENPAWCGVAIMVNLINISPEDP
490309833_Burkholderia_mallei	QTLPLPIDLGSRLAGEQMLAESGARSAYASLGSHEFVTQKTQSYCGVALVMVLNALVPPAAE
427714658_Syzygium_melanosporum_PCC6312	QPLPLPIDLGSRLAGEQMLAESGARSAYASLGSHEFVTQKTQSYCGVALVMVLNALVPPAAE
AB578257_Coxiella_burnetii	QTLTSLTSLRTGESLLESQAKADVLPLMSHFVTQINPAYCGVASVMVLNALVTTANT
209364092_Coxiella_burnetii5J108111	TLLPVPVIAFNSPAGIKLFNKSQHKTAFWELIPIYFTTEKGVAFCGVASVMVLNAVIKPIPT
145588734_Polynucleobacter_necessarius	LTNAFPVYINTTAGSERLLHSTHNRQYFALASVYDTERVTCGIMSATINSLVVPITP
497514158_Rhodobacteraceae_bacteriumHTCC2083	APVAAQLYLITTEAGERDLLGAELNDRYFSLASYLEYEQVLTFCGPAVASAVANSLIDPSPD

	61	71	81	91	101	111
EgPCS1						
ACC812122_Nostoc_punctiforme	WKAP	PWRW	FSEPIEYAK	RDFINFCICEANGARARFQKHVMVVSYSRSSLKQTGGHYSPIGG		
Q9S7Z3_Arabidopsis_thaliana	QYKPYRVFTQTPEVVSRQ	GMTLKL	LLSSYGKVVHFRKQAILINYL	RKEIGQEKGHISPVAA		
ACJ71777_Leucaena_leucocephala	WKGP	PWRW	FDEPLEVVK	KEGISFCLAHCSGAKVFRFRKFMISTYHRGVFKQTGGHFSPIGG		
BAJ34584_Thellungiella_halophila	WKGP	PWRW	FDEPLEVVK	KEGISFCLAHCSGAKVFRFRKLMISTYHRGVFKQTGGHFSPIGG		
AEW23125_Amaranthus_tricolor	WKGP	PWRW	FDEPLEVVK	KEGISFCLAHCSGAKVFRFRKFMISTYHRGVFKQTGGHFSPIGG		
BAB85602_Brassica_junceae	WKGP	PWRW	FDEPLEAVKE	KGISFCLAHCSGAKVFRFRKFMISTYHRGVFKQTGGHFSPIGG		
BAB93120_Thlaspi_caerulescens	WKGP	PWRW	FDEPLEVVK	DKGISFCLAHCSGAKVHFRNFVMISTYHREIFKQTGGHFSPIGG		
BAB93119_Thlaspi_japonicum	WKGP	PWRW	FDEPLEVVK	DKGISFCLAHCSGAKVFRNFVMISTYHREIFKQTGGHFSPIGG		
ACL80669_Noccaea_caerulescens	WKGP	PWRW	FDEPLEVVK	DKGISFCLAHCSGAKVFRDFVMISTYHRGVFEQTGGHFSPIGG		
NP_171894_Arabidopsis_thaliana_PCS2	WKGP	PWRW	FDEPLEIVK	DKGISFCLAHSSGAKVFRFRKYMISTYHRQVLKQTGGHFSPIGG		
ADZ24787_Arabidopsis_halleri_PCS2	WKGP	PWRW	FDEPLEIVK	DKGISFCLAHSSGAKVFRFRKYMISTYHREVLKQTGGHFSPIGG		
XP_002892190_Arabidopsis_lyrata_PCS2	WKGP	PWRW	FDEPLEIVK	DKGISFCLAHSSGAKVFRFRKYMISTYHRQVLKQTGGHFSPIGG		
ABY89660_Noccaea_caerulescens	WKGP	PWRW	FDEPLEKVK	DKGISFCLAHSSGAKVFRFRKVMISTYHRGVFKQTGGHFSPIGG		
AAO74500_Nicotiana_tabacum	WKGP	PWRW	FDEPLEKVK	AKGISFCLAHCSGAKVFRFRKQVLISSYHRLFKQTGGHFSPIGG		
AA582881_Sesbania_rostrata	WKGP	PWRW	FDEPLEKIK	VRGISFCLAHCSGAKVHFRKYVVISSYHRGALKQTGGHFSPIGG		
NP_001235576_Glycine_max_homoPCS	WKGP	PWRW	FDEPLETVK	ARGITFCLAHCSGAKVHFRKYVVISSYHRAALKQTGGHFSPIGG		
AEY68568_Pyrus_betulifolia	WKGP	PWRW	FDEPLETVK	KEGISFCLAHCSGAKVFRFRKYVVISSYDRSVLKQTGGHFSPIGG		
AAU93349_Lactuca_sativa	WKGP	PWRW	FDEPLEKVK	AKGISFCLAHCSGAKVFRFRKHVVISSYNRATFKQTGGHFSPIGS		
ACU44656_Sonchus_arsensis	WKGP	PWRW	FDEPLEKVK	AKGISFCLAHCSGAKVFRFRKHVVISSYNRATFKQTGGHFSPIGG		
AAD50592_Triticum_aestivum	WKGP	PWRW	FDEPLEHKV	KAEGITFCLAHCSGAKVFRFRHLLISSYHRSPPFKQTGGHFSPIGG		
BAJ85525_Hordeum_vulgare	WKGP	PWRW	FDEPLEHKV	KAEGITFCLAHCSGAKVFRFRHLLISSYHRSPPFKQTGGHFSPIGG		
XP_002454970_Sorghum_bicolor	WKGP	PWRW	FDEPLEDKV	KDQGITFCLAHCSGADVRLRRHLLIASYHRKAFKQTGGHFSPIGG		
AAO13810_Cynodon_dactylon	WKGP	PWRW	FDEPLEDKV	KAQGITFLLAHCSGADVRLRRHLLIASYHRKAFKQTGGHFSPIGG		
NP_001056534_Oryza_sativa	WKGP	PWRW	FDEPLEDKV	KAEGITFCLAHCSGANVFRFRHLLIASYHRKPFKQTGGHFSPIGG		
XP_002320627_Populus_trichocarpa	WKGP	PWRW	FDDPLVKI	KEKGITFCLAHCSNAKVRFRFRFVISSYHRGAFKQTGGHFSPIGG		
XP_00352130_Glycine_max	WKGP	PWRW	FDEPLAKV	LEGITFCLARCSGAKVFRFRNRVVISYHRTPLNQTGGHFSPIGG		
AFK36941_Lotus_japonicus	WKGP	PWRW	FDDPLEKI	VKQGITFCLARCSGADVFRDRVLISYHRSGLKQTGGHFSPIGG		
BAF75863_Fagopyrum_esculentum	WKGP	PWRW	FDDPLEKVK	VTGISFCLAHCSGAEVHFRSVYLISYHRSGLKQTGGHFSPIGG		
XP_002973139_Selaginella_moellendorffii	WKGP	PWRW	FDEPLEKVK	TDGISFCLGECSGARVFRFSVLLIASYNRQLKQTGGHFSPIGG		
105477_SelaginellaGI	WKGP	PWRW	FDEPLEKVK	TDGISFCLGECSGARVFRFSVLLIASYNRQLKQTGGHFSPIGG		
A816U6_Chlamydomonas_reinhardtii	WKGS	WRWF	DEPLEDAV	KEEGITFCLARCSGANVFRFRFREVIVVSYSRKAFKQTGGHFSPIGG		
302847090_Volvox_carteri	WKGS	WRWF	DEPLEDAV	KEEGITFCLARCSGANVFRFRAEVIVVSYSRKAFKQTGGHFSPIGG		
136723_Chlorella_variabilis	WKGP	PWRW	FHEPLEPKV	REEGITFCLARCSGANVFRFRGMVIVVSYSRQEFKQTGGHFSPIGG		
384245625_Coccomyxa_subellipsoidea	WKGP	PWRW	FHEPILRVA	EEGI--CLARCSGANVFRFREDVLISYSRKQFLQTGGHFSPIGG		
302843401_Volvox_carteri	WKGAW	RWSE	SESMEEV	KEEGITFCLARCSGANVFRFRRLLMVVSYSRKAFKQSGGHFSPIGG		
BAB64932_Athyrium_yokoscense	WKGP	PWRW	FDEPLEKVK	KNGITFCLAQCSGASVFRFSFVLISYDRFRPLKQTGGHFSPIGG		
ADR51694_Pteris_vittata	WKGP	PWRW	FDEPLENV	KKNGITFCLAQCSGASVFRFSFVLIVSYDRQILKQTGGHFSPIGG		
428304353_Crinalium_episammum_PCC9333	WKG	VWRW	YGEPLPVV	KEEGITFCLARCSGANVFRFRQTIIIVSYSRQILGQTGGHFSPIGG		
428304353_Crinalium_episammum_2PCC9333	WKG	VWRW	YGEPLPVV	KEEGITFCLARCSGANVFRFRQTIIIVSYSRQILGQTGGHFSPIGG		
485640797_Emiliana_huxleyi_CCMF1516	WKGP	PWRW	FHEPLEDIRR	RGITFCLARCSGAACV---ALVVSYSRREFKQTGGHFSPIGG		
ZP_08492182_Microcoleus_vaginatus	WKG	VWRW	YGEPLSVI	KENGITFCIARCSGANVFRFRQAVLVVSYSRKVLGQTGGHFSPIGG		
'428317750_Oscillatoria_PCC_7112'	WKG	VWRW	YGEPLSVI	KENGITFCIARCSGANVFRFRQAVLVVSYSRKVLGQTGGHFSPIGG		
ZP_05027234_Microcoleus_chthonoplastes	WRGV	VWRW	YGESL	PVIQETGITLCISRCNGAKVFRFRQDVMVAAYSRKVLGQTGGHFSPIGG		
493032501_Coleofasciculus_chthonoplastes	WRGV	VWRW	YGESL	PVIQETGITLCISRCNGAKVFRFRQDVMVAAYSRKVLGQTGGHFSPIGG		
428225164_Geitlerinema_PCC7407	WRGV	VWRW	YGEPLSTI	QQQGISMCIARCSGAQVFRFRETLMVVSYSRQVLGQTGGHFSPIGG		
470517486_Acanthamoeba_castellanii	WKGP	PWRW	FSESLE	LVKKQGITFCLARCSGAQVFRNVVTIVSYNRGKVGQTGGHFSPIGG		
XP_635353_Dictyostelium discoideum	WKG	PWRW	FPAEP	IESVKKRGITFCLSKCSGANIRFRNSILVMVSYSRKVLGQTGGHFSPIGG		
330794008_Dictyostelium_purpureum	WKGP	PWRW	FPAEP	IDSVKKRGITFCLSKCSGANIRFRNSILVMVSYSRKVLGQTGGHFSPIGG		
EFA77058_Polysphondylium_pallidum	WKG	SWRW	VFVETRETI	TRRGMTFCLSRCSGASVFRFREFLLVLCYDRKSLGQVGGHYSPIGG		
323451775_Aureococcus_anophagefferens	WKG	VWRW	FDEPLVD	VEKEGIVLCLARCSGLEARFRDAVLAAYSRKVLGQTGGHFSPIGG		
ABR13683_Eisenia_fetida	WKGP	PWRW	FHEP	INVIKSGITFCLAVCNTLNVFRQQLIVIVSYSRKGLDQTGGHFSPIAG		
NP_593552_Schizosaccharomyces_pombe	WKG	SWRW	YDQSLSD	IEKDGVTCLANCSGLRTRCFRKDVMAISFCRKVLGQTGGHFSPIGG		
XP_002175610_Schizosaccharomyces_japonicus	WKGAW	RWYDQSL	SNIKD	GITICLAACNGLSARFRRLMAVSYSRKALGQTGGHFSPIGG		
EIM19133_Walleimia_sebi	WKGP	PWRW	FYEP	ISLVEHEGITFCLAECSNIEITKFRKVLILSYSRKTVNQTGGHFSPIGG		
EKC27807_Crassostrea_gigas	WKGP	PWRW	FHEP	ISLVEHEGITFCLAECSNIEITKFRKVLILSYSRKTVNQTGGHFSPIGG		
XP_002156317_Hydra_magnipapillata	WKGP	PWRW	FHYHES	LDLVQQNGITCLATCNNLDRFDVLTCSYSRKSVLNQTGGHFSPIGA		
AAK62991_Caenorhabditis_elegans	WKAP	PWRF	FYHEPLENIRK	SGINLCLACNRKLSYFRTSLLVASYDRSVLGQTGGHFSPLAA		
EGT44842_Caenorhabditis_brenneri	WKAP	PWRF	FYHEPLENIRK	SGINLCLATCNRLKSYFRKSLLVASYDRSVLGQTGGHFSPLAA		
XP_001902100_Brugia_malayi	WKSP	PWRF	FHEPLDDIK	KTGITCLAECSNKLTYLRENVLVSYNRQVLGQTGGHFSPLGA		
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348672680_Phytophthora_sojae	WKGP	PWRW	FSES	LSVAKEKGISMCLARCSGVHTRFRDIVVVLNYSRKVLGQTGGHFSPIGG		
XP_002128372_Ciona_intestinales	WKG	VWRW	FHES	LEDVQRQGVTCIAKCNQLNAYFRELVVVICSFGRRELQTGGHFSPIGG		
EJK76788_Thalassiosira_oceanica	WKGP	PWRW	FYEEDIEE	VKKTGINFCLAKCQGLTVYFRNVVLISYTRKVLSTQGHHFSPIGA		
XP_002569764_Schistosoma_mansoni	WKGP	PWRW	FHEPRD-V	LTKGITLCIARCYGLDVRFQDVLVVCYSRSALGQTGGHFAPIVGG		
CMI111C_Cyanidioschyzon_merolae	WKGP	PWRW	FSEPLHIV	AREGITCLGQCNGALVQFRESLLVLCYAREALQQTGGHFSPIAA		
XP_002838367_Tuber_melanosporum	WRKP	PWRW	FPTQPLEVVK	KEGITCLACKNGLEAFFHQAVMAVSYSRASLGQTGGHFSPIVGG		
BAB72932_1_Nostoc	QYSPYRVFTQAEVVVARQ	GMTLRL	LIASYGKVVHFRKQVVI	VNYLRKEIGQERGHISPLAA		
ABA22569_Anabaena_variabilis	QYSPYRVFTQAEVVVASQ	GMTLGL	LIASYGVEVHFRKQVVI	VNYLRKEIGQERGHISPLAA		
434384310_Chamaesiphon_minutus_PCC6605	QHKPFVRFTQSPDIVSK	RGMTLGL	LASHGAKVHFRSL	LAIIVNYLRKEIGQERGHISPIAA		
307153464_Cyanotheca_PCC7822	VHKPYHAFTQTAEQVS	RQGMTLQL	LESYSLKVYFRQLVVI	VNYLRSTLHQRERGHISPIAA		
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209364092_Coxiella_burnetii_5J108111	SHSPFFYIFNQTPAEINS	KGATLQSLKTFN	IFVHFRRLAI	IVNFCRKYIKEQGGHFSPLAA		
145588734_Polynucoleobacter_necessarius	ELAPHYFNQTPAEINS	KGATLQSLKTFN	IFVHFRRLAI	IVNFCRKYIKEQGGHFSPLAA		
497514158_Rhodobacteraceae_bacteriumHTCC2083	RLYPFWTLFTQPYGM	VEHEGLTLQFIEN	LGLAFLREMILIVNYSR	QALPQTGGHISPIGA		

	121	131	141	151
EgPCS1				
ACC81212_Nostoc_punctiforme	YQLVLVMDVARFKYPPHWVPLDALWSRGYVLLS			
Q9S7Z3_Arabidopsis_thaliana	YDRFLILDVSRSKYPPVWVKTTDLWSRGFVFVS			
ACJ71777_Leucaena_leucocephala	YDMALILDVARFKYPPHWVPLKLLWRRGFMLIS			
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AEW23125_Amaranthus_tricolor	YDMALILDVARFKYPPHWVPLKLLWRRGFMLIS			
BAB85602_Brassica_juncea	YDMALILDVARFKYPPHWVPLKLLWRRGFMLIS			
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AAV82881_Sesbania_rostrata	YDMALILDVARFKYPPHWVPLTLLWSRGFMILIS			
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AAU93349_Lactuca_sativa	YDMALILDVARFKYPPHWVPLLWRRGFMLIS			
ACU44656_Sonchus_oleraceus	YDMALILDVARFKYPPHWVPLKLLWRRGFMLIS			
AAD50592_Triticum_aestivum	YDMALILDVARFKYPPHWVPLTLLWLRGFMILVS			
BAJ85525_Hordeum_vulgare	YDMALILDVARFKYPPHWVPLTLLWLRGFMILVS			
XP_002454970_Sorghum_bicolor	YDMALILDVARFKYPPHWVPLQLLWIRGFMILIS			
AAO13810_Cynodon_dactylon	YDMALILDVARFKYPPHWVPLSLLWLRGFMILIS			
NP_001056534_Oryza_sativa	YDMALILDVARFKYPPHWVPLPLLWLRGFMILIS			
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XP_00352130_Glycine_max	YDMVLILDVARFKYPPHWVPLTLLWRRGYMIIS			
AFK36941_Lotus_japonicus	YDMVLILDVTRYKYPPHWVPLTLLWQRGHMIIS			
BAF75863_Fagopyrum_esculentum	YDLVLVLDVARFKYPPHWVPLTLLWRRGFMLILA			
XP_002973139_Selaginella_moellendorffii	YDMALILDVARFKYPPHWVPLNLVWSRGFMVVT			
105477_SelaginellaGJI	YDMALILDVARFKYPPHWVPLNLVWSRGFMVVT			
A8I6U6_Chlamydomonas_reinhardtii	YDLVLVLDVARFKYPPHWVPLPM-----			
302847090_Volvox_carteri	YDLVLVLDVARFKYPPHWVPL-----			
136723_Chlorella_variabilis	YDLALILDVARFKYPPHWVPLMLPYRGMFRMG			
384245625_Coccomyxa_subellipsoidea	YDLVLILDVARFKYPPHWVPLTELFPRGYMKMG			
302843401_Volvox_carteri	YDVVLLLDVARFKYCPHWVAVDELLWGGDGGWG			
BAB64932_Athyrium_yokoscense	YDMALILDVARFKYPPHWVPLSLLWSRGFMIIY			
ADR51694_Pteris_vittata	YDMVLILDVARFKYPPHWVPLSLLWSRGFMIIIT			
428304353_Crinalium_epipsammumPCC9333	YDLVLILDVARFKYPPHWVPLPLLWGRGYMLLS			
428304353_Crinalium_epipsammum_2PCC9333	YDLVLILDVARFKYPPHWVPLPLLWGRGYMLLS			
485640797_Emiliania_huxleyiCCMP1516	YDSVLLLDVARFKYPPHWVGLRALWTRGYLLLS			
ZP_08492182_Microcoleus_vaginatus	YDLVLILDVARFKYPPHSVSLPLLWCRGYILLQ			
'428317750_Oscillatoria_PCC_7112'	YDLVLILDVARFKYPPHWVSLPLLWCRGYISLQ			
XP_05027234_Microcoleus_chthonoplastes	YDLVLILDVARFKYPPHWVPLPLLWSRGYILLQ			
493032501_Coleoelasciculus_chthonoplastes	YDLVLILDVARFKYPPHWVPLPLLWSRGYILLQ			
428225164_Geitlerinema_PCC7407	YDLVLILDVARFKYPPHWVPSLLWSRGYVLVQ			
470517486_Acanthamoeba_castellanii	YDMVLIMEVARFKYPPHWVPLSMLFSRGFVTLIS			
XP_635353_Dictyostelium_discoideum	YDLALVLDVARFKYSPHWVPEVLWPRGYLLIS			
330794008_Dictyostelium_purpureum	YDLALVLDVARFKYAPHWVPVETLWPRGYVVLIS			
EFA77058_Polysphondylium_pallidum	YDLALVMDVARFKYPTYYVVPVKALWTRGFYIIS			
323451775_Aureococcus_anophagefferens	YDAVLLLDVARFKYPPHWVPLPLLPRGWIRLT			
ABR13683_Eisenia_fetida	YDLVLIMDVARFKYQPHWVKHSLFSRGYLLLS			
NP_593552_Schizosaccharomyces_pombe	FNKILILDVARFKYPCYWVDLKLMPYRGYVLLS			
XP_002175610_Schizosaccharomyces_japonicus	IDKVLILDVARFKYPSYVWVDSKLLYPRGYVILR			
EIM19133_Wallmania_sebi	YDSLILLDVARFKYPSYVWTSIEDAYPRGYTLLS			
EKC27807_Crassostrea_gigas	YDLILILDVARFKYPPHWISLELLWPRGYLSVR			
XP_002156317_Hydra_magnipapillata	YDMVLIFDVARFKYPPHWVKLDLLWPRGYIVLR			
AAK62991_Caenorhabditis_elegans	YDQVLIMDVARFKYPPHWVKLETLQPRGLVELE			
EGT44842_Caenorhabditis_brenneri	YDQVLIMDVARFKYPPHWVKLETLQPRGFIELE			
XP_001902100_Brugia_malayi	YDQILIMDVARFKYPPHWVPLTILRPRGYLLLK			
XP_002672798_Naegleria_gruberi	YDMVLVLDVARFKYPPHWVKLDLLWSRGFVVIK			
XP_002997994_Phytophthora_infestans	YDMVLLMDVARFKYPPHWVKISRVYPRGLVLVK			
348672680_Phytophthora_sojae	YDMVLLMDVARFKYPPHWVKLSMVFPRLVLILR			
XP_002128372_Ciona_intestinales	YDVVLVLEPARFKYPPYWLISLEALWPRGYMILE			
EJK76788_Thalassiosira_oceanica	YDRVVLDTARFKYGLHWVKPLLLFSRGYMTLS			
XP_002569764_Schistosoma_mansoni	YELVFLFDARFKYPPHWVSLTKLWPRGFVVLIR			
CMT111C_Cyanidoschyzon_merolae	YDRALILDVARFKYPPHWVPLPDLYPRGLAMVR			
XP_002838367_Tuber_melanosporum	YGMVLILDVARFKYPSYVWPIRMLFPRGYSMLR			
BAB72932_1_Nostoc	YDRFLIMDVSRKYPPVWVKTTDLWTRGFVFVS			
ABA22569_Anabaena_variabilis	YDRFLIMDVSRKYPPVWVKTTDLWTRGFVLVS			
434384310_Chamaesiphon_minutusPCC6605	YDRFLILDVSRKYPPVWVKAVDLWTRGFVLVT			
307153464_Cyanospora_PCC7822	YDRFLILDVARYKYPPVWVVSASELWSRGFVLVN			
492895672_Burkholderia_thailandensis	YDRFLILDVSRKYPPVWVTTADLYSRGYVLID			
490309833_Burkholderia_mallei	YDRFLILDVSRKYPPVWVTTADLYSRGYVLID			
427714658_Synechococcus_PCC6312	YDQVLILDVARYKYPIWVSVQALWSRGFVLIR			
ABS78257_Coxiella_burnetii	YDRFLLLDVARYKYLPMVWIKTDELYSRGFIIVS			
209364092_Coxiella_burnetii5J108111	YDRFLLLDVARYKYLPMVWIKTDELYSRGFIIVS			
145588734_Polynucleobacter_necessarius	YDSVLILDVAKYKPPFVWTTSDLLSRGIVQIT			
497514158_Rhodobacteraceae_bacteriumHTCC2083	YDSVLILDVAKYKPPFVWISTQKLHSRGFVQVS			

Table S4. PC₂ synthesis by *EgPCS* in the presence of *S*-methyl-GS, N-acetylcysteine (NAC), oxidized glutathione (GSSG), H₂O₂, cumene hydroperoxide (CHP), or *tert*-butoxyl hydroperoxide (t-BHP), and 40 mM GSH and 50 μM Cd²⁺ or Zn²⁺ for 30 min. 100% values represent 5–10 and 14–22 μmol x min⁻¹ x mg_{protein}⁻¹ with Cd²⁺ and Zn²⁺, respectively. ‡

Experiments carried out without β-mercaptoethanol. * *EgPCS* (100 μg mL⁻¹) was pre-incubated with the indicated peroxide for 1 h at room temperature; thereafter an enzyme aliquot (0.4 μg mL⁻¹) was used to determine the activity.

		% PC ₂ synthesis	
		Cd ²⁺	Zn ²⁺
Control		100	100
S-methyl-GS	20 mM	49 (2)	43 ± 2 (5)
	40 mM	34 ± 19 (3)	24 ± 3 (5)
NAC	40 mM	78 ± 10 (3)	91 ± 4 (3)
‡GSSG	0.1 mM	87 ± 15 (3)	89 ± 5 (3)
*H ₂ O ₂	0.1 mM	37 ± 10 (4)	43 ± 3 (4)
*CHP	0.1 mM	23 ± 9 (4)	28 ± 4 (4)
*t-BHP	0.1 mM	77 ± 16 (3)	76 ± 2 (4)

Supplementary Figures

Figure S1: Acyl γ -EC-*Eg*PCS enzyme intermediate was modeled to indicate the correct orientation of Cys-70, His-179 and Asp-197 using the *Ns*PCS crystal structure as scaffold (see Experimental section).

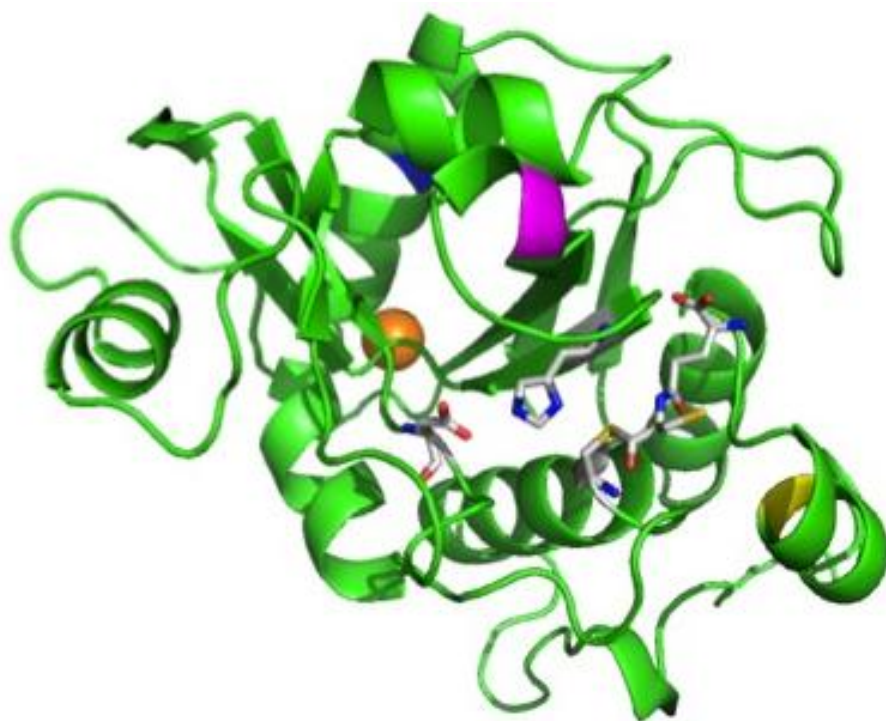
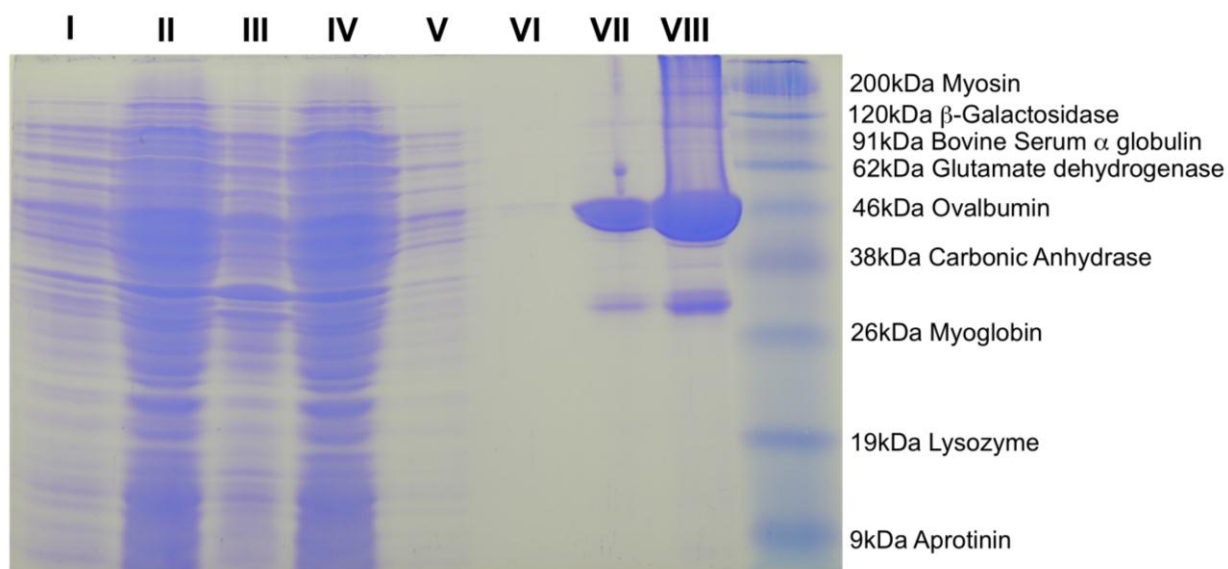


Figure S2: Representative gel showing the purification process of recombinant *EgPCS*.

The purity of this preparation was 79%.



I .- BL21 cells (transformed with *EgPCS*) induced with IPTG (1:4 v/v)

II .- Supernatant from lysate BL21 cells (1:4 v/v)

III .- Precipitated from lysated BL21 cells (1:4 v/v)

IV .- Flow through (1:4 v/v)

V .- Column wash # 1 (using 5 mM imidazole) (1:4 v/v)

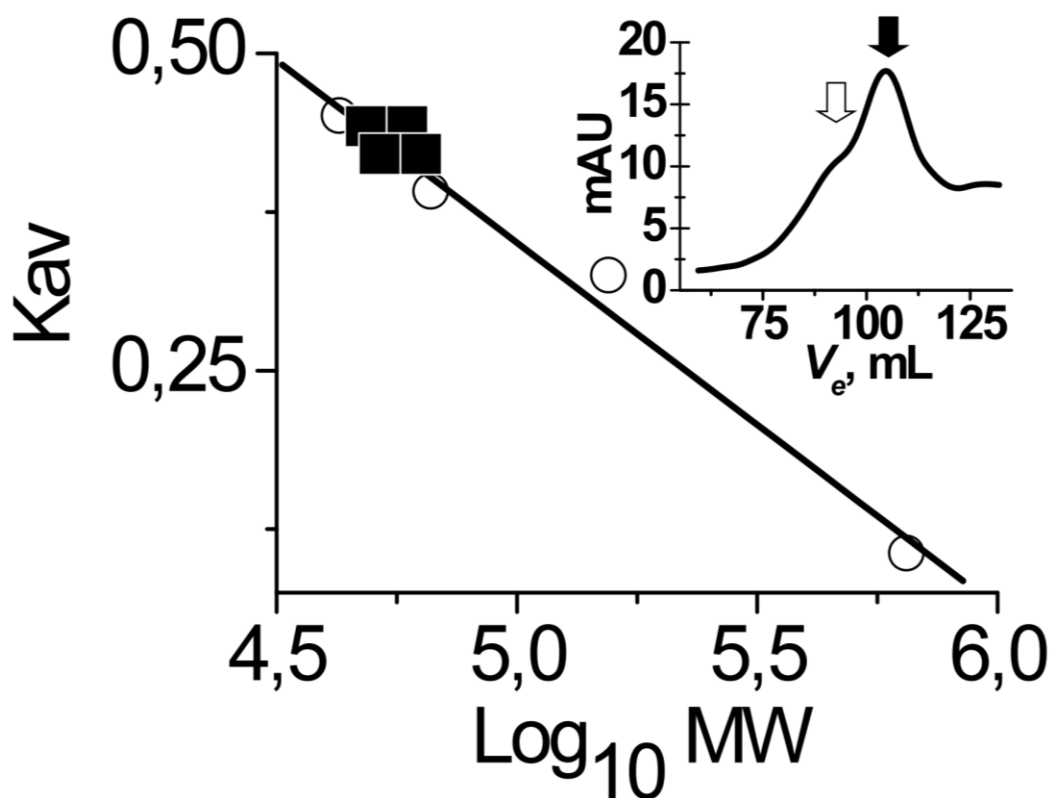
VI .- Column wash # 4 (using 10 mM imidazole) (1:4 v/v)

VII .- *EgPCS* (eluted with 100 mM imidazole) (1:20 v/v)

VIII .- *EgPCS* (eluted with 100mM imidazole) (concentrated)

Figure S3: Molecular exclusion chromatography of *EgPCS*.

Empty circles represent the molecular weight calibration curve described in the Experimental section; and black squares represent the molecular weight determined, separately, for four independent *EgPCS* preparations ($1\text{--}1.5\text{ mg mL}^{-1}$). Inset shows a typical molecular exclusion chromatography profile of an *EgPCS* preparation; black arrow indicates the peak that corresponds to monomeric *EgPCS*; while white arrow indicates the shoulder corresponding to the presence of dimeric *EgPCS*. V_e = elution volume.



K_{av} was calculated as $K_{av} = (V_e \times MW_{EB}) / (V_t \times MW_{EB})$

Where:

V_e = (peak fraction) $\times$ (fraction volume)

MW_{EB} = molecular weight of dextran blue (68.7 KDa)

V_t = (fraction volume) $\times$ (column diameter / 2)² $\times$ (column length)

Fraction volume = 3.3 mL; column diameter = 1.5 cm; column length = 85.8 cm

Figure S4: MS of *EgPCS* reaction products using γ -EC (A) and PC_2 (B) as substrate. Both experiments were carried out in the presence of $50\ \mu\text{M}\ \text{Cd}^{2+}$.

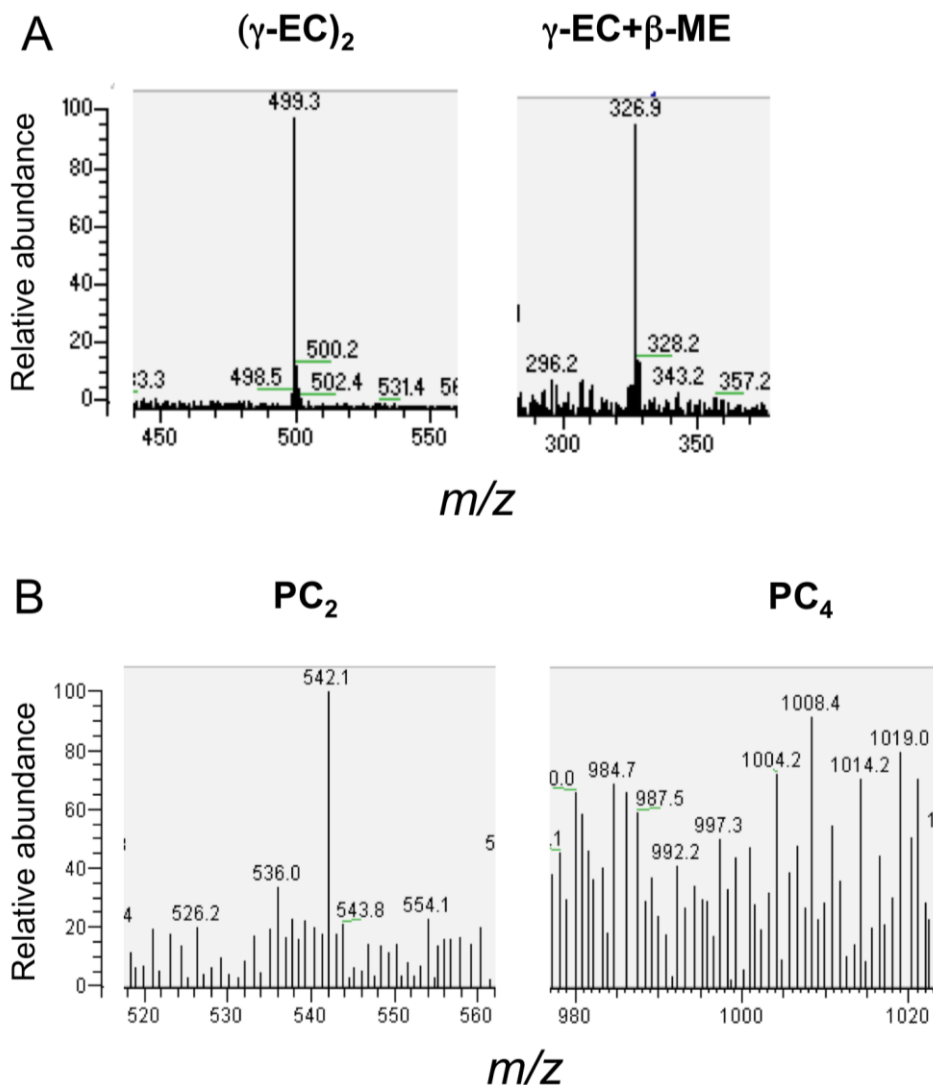


Figure S5: Time course of PC₂ (■) and PC₃ (○) synthesis by *EgPCS* (4 μg mL⁻¹) was allowed to proceed for 24 h using 40 mM GSH as substrate and 50 μM Cd²⁺.

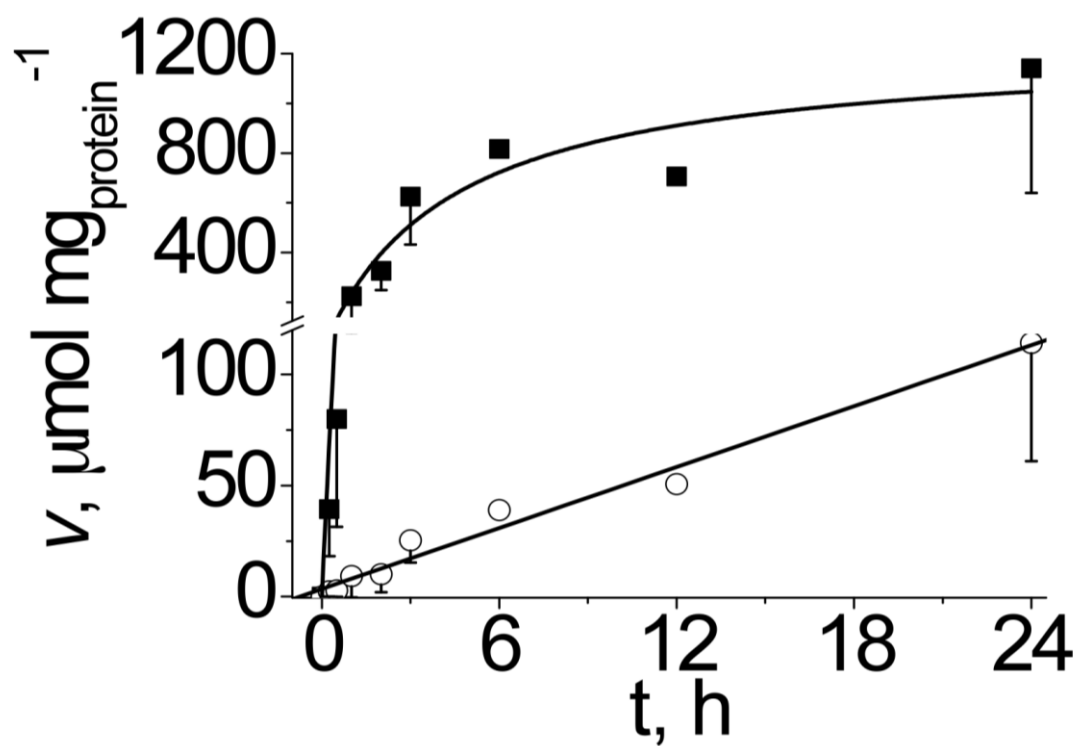


Figure S6: MS of *EgPCS* ($4\ \mu\text{g mL}^{-1}$) reaction products using *S*-methyl-GS as substrate, and in presence of 0.1 mM EGTA (A), or 50 $\mu\text{M Cd}^{2+}$ (B). *S*-methyl-GS (322 m/z), *S*-methyl-PC₂ (568 m/z), *S*-methyl-PC₃ (814 m/z) and *S*-methyl-PC₄ (1,060 m/z) are indicated on the MS profiles.

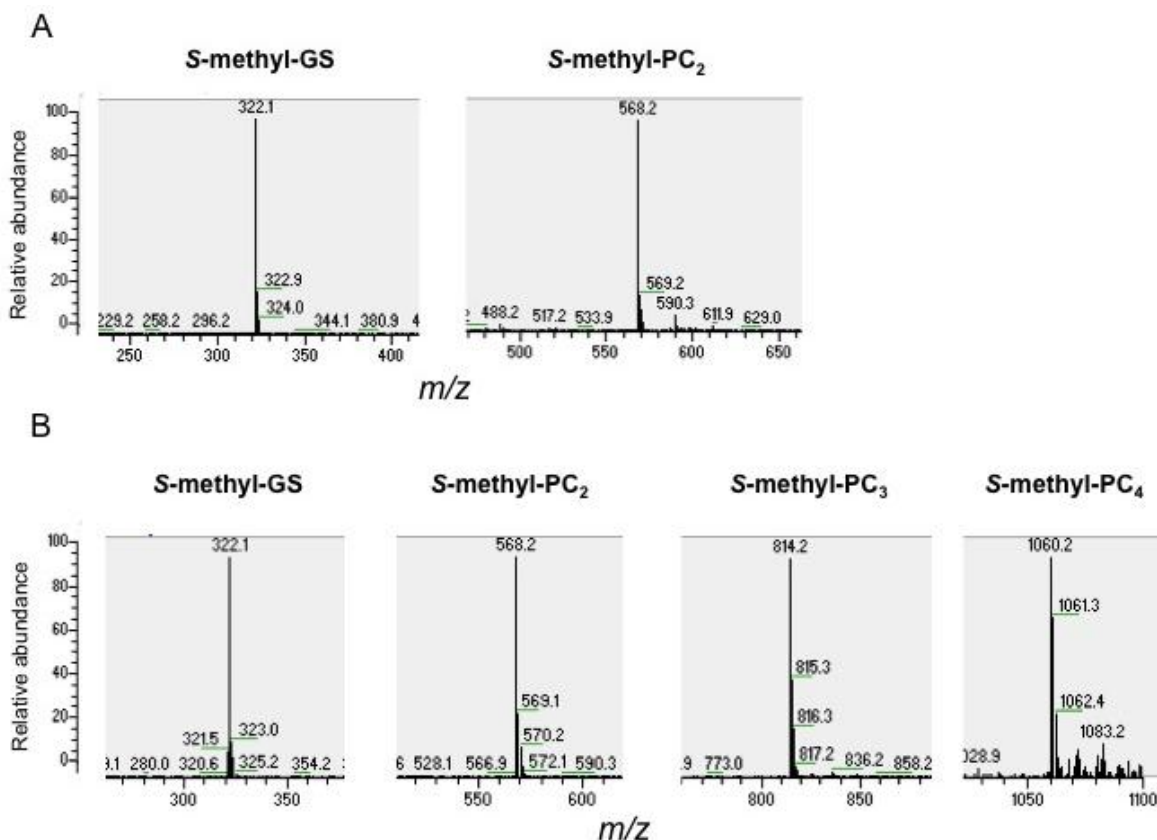


Figure S7: Formation of PC₂ and hybrid dipeptide GSH/S-methyl-GS. HPLC profiles of the *EgPCS* reaction products with 40 mM GSH, 50 μM Cd²⁺ (gray line), and 20 (black dotted line) or 40 mM (black solid line) S-methyl-GS.

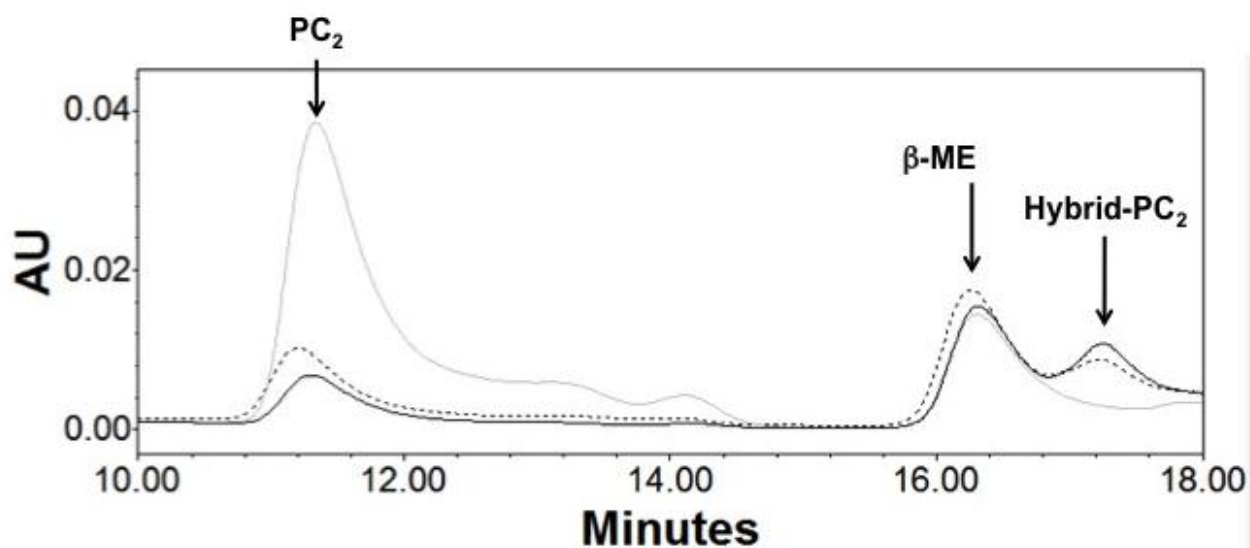


Figure S8: Non-linear regression analysis of data from Figs. 4A (A) and 4B (B) fitted to the constant *plus* Hill equation using the Origin Microcal v. 7.5 software. Tables under each figure show the kinetic parameters obtained.

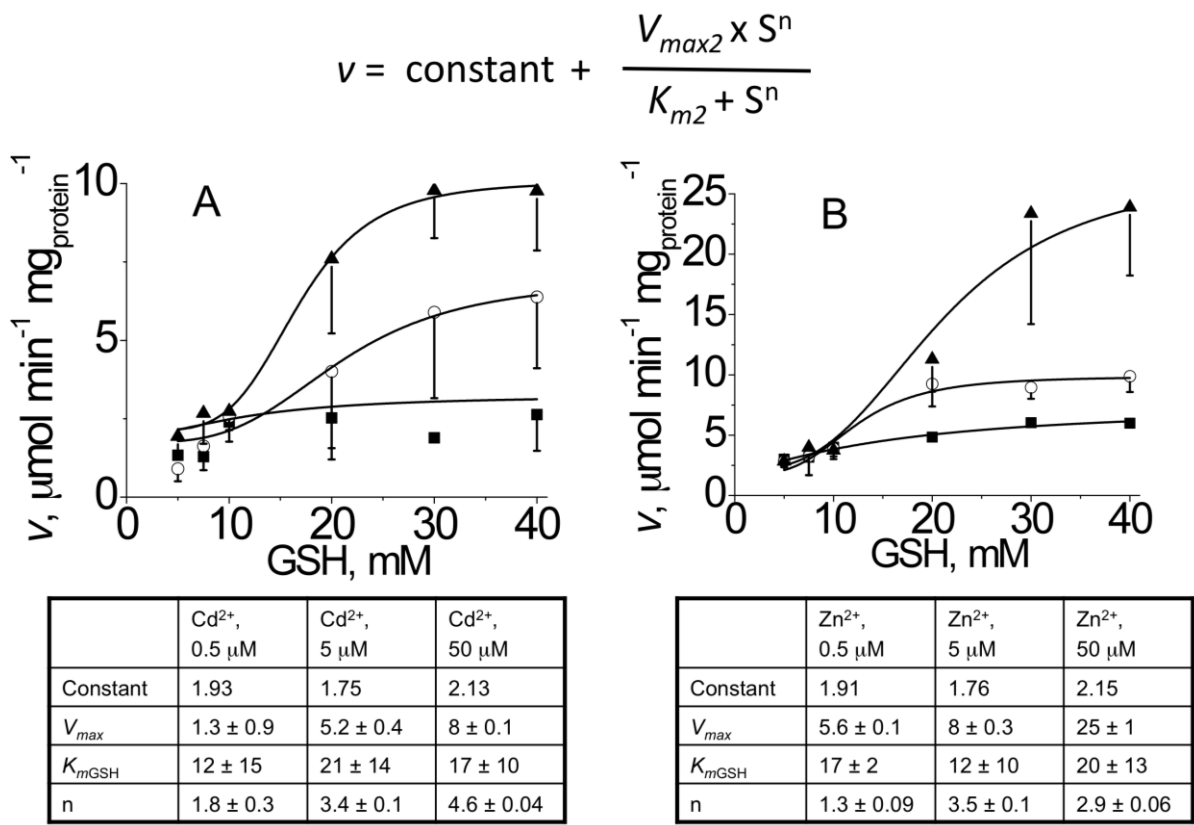


Fig.S9: Formation of PC_2 by *EgPCS* (dotted line) but not by *EgPCS_Nter*_{235aa} (solid line) after incubation with 40 mM GSH and 50 μ M Cd^{2+} for 24 h.

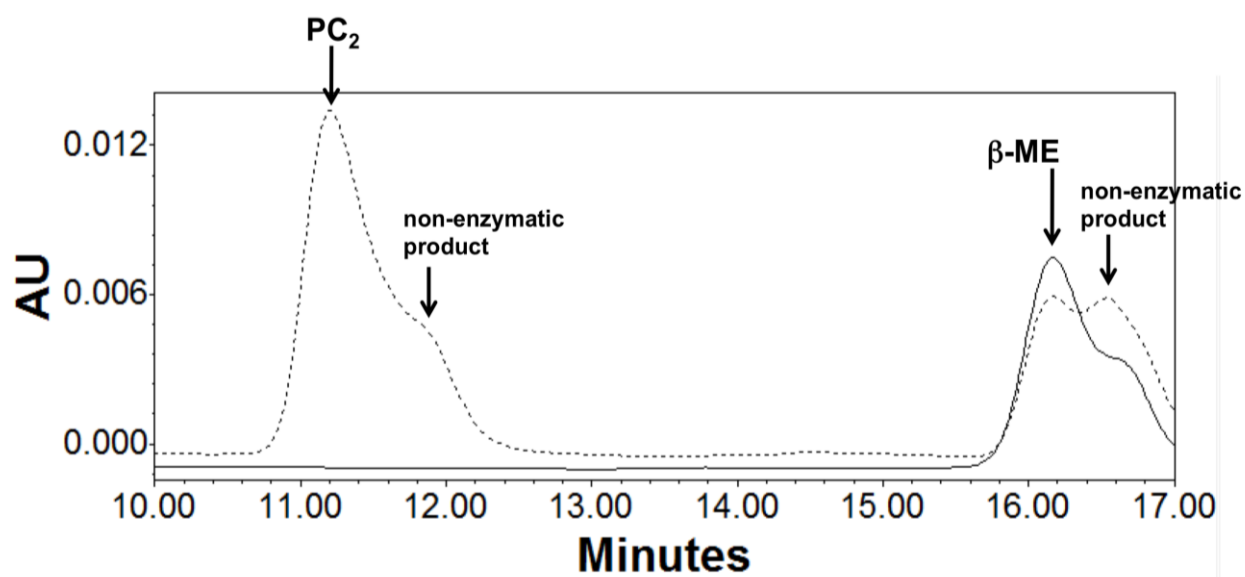


Figure S10: Expression of *EgPCS* determined by end-point RT-PCR (A) in *E. gracilis* cells exposed to 50 μM Cd^{2+} after 6, 12, 24, and 48 h. Actin gene was used as housekeeping, loading control. A representative experiment is shown, of four made, all which exhibited similar results. (B) Real-Time PCR analysis of a culture monitored at the indicated times. The relative abundance was calculated by using Equation (2), in which PCR efficiency (1.94 - 2) was determined by LingRegPCR software version 7.5 (Dr. J.M. Ruijter, Department of Anatomy and Embriology, Academic Medical Center, Amsterdam, The Netherlands); the $\Delta\Delta\text{Ct}$ was calculated respect to actin and non-exposed cells at each time..

$$\text{Relative abundance} = (\text{PCR efficiency})^{-(\Delta\Delta\text{Ct})} \tag{2}$$

