

Supplementary Material

The 1.4 Å resolution structure of *Paracoccus pantotrophus* pseudoazurin

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Tables

Table S1 - Statistics for the structural-based sequence comparison, as shown in Figure 4, for pseudoazurins from different organisms and, with some plastocyanins and amicyanins, another type 1 copper proteins.

PDB code	Smith-Waterman score	%-tage identity	a.a. overlap	Seq. length	z-score	Protein name and Source
<u>3ERX</u>	-	-	-	123	-	Pseudoazurin <i>Paracoccus pantotrophus</i>
<u>1BQK</u>	587	69.2%	120	124	740.9	Pseudoazurin <i>Achromobacter cycloclastes</i>
<u>2UX6</u>	540	66.9%	121	122	682.6	Pseudoazurin <i>Achromobacter cycloclastes</i> with engineered amicyanin ligand loop
<u>1PAZ</u>	537	62.4%	117	120	679.0	Pseudoazurin <i>Alcaligenes faecalis</i>
<u>1PMY</u>	401	48.3%	118	123	510.0	Pseudoazurin <i>Methylobacterium extorquens</i>
<u>3EF4</u>	402	49.6%	123	123	501.3	Pseudoazurin <i>Hyphomicrobium denitrificans</i>
<u>1PCS</u>	171	39.8%	88	98	226.0	Plastocyanin <i>Cyanobacterium synechocystis</i>
<u>1M9W</u>	168	39.8%	88	98	222.2	Plastocyanin <i>Cyanobacterium synechococcus</i> sp.
<u>2PLT</u>	165	32.6%	89	98	218.5	Plastocyanin <i>Chlamydomonas reinhardtii</i>
<u>1IUZ</u>	158	31.1%	90	98	209.8	Plastocyanin <i>Ulva arasakii</i>
<u>7PCY</u>	146	28.9%	90	98	194.9	Plastocyanin <i>Enteromorpha prolifera</i>
<u>1PLA</u>	142	32.6%	89	97	190.0	Plastocyanin <i>Petroselinum crispum</i>
<u>1BXU</u>	161	33.7%	83	91	214.1	Oxidized plastocyanin from <i>Synechococcus</i> sp.
<u>1B3I</u>	139	35.6%	87	97	186.3	Plastocyanin <i>Prochlorothrix hollandica</i>
<u>9PCY</u>	145	32.3%	96	99	173.7	Plastocyanin <i>Phaseolus vulgaris</i>
<u>1AG6</u>	155	33.7%	98	99	168.8	Plastocyanin <i>Spinacia oleracea</i>
<u>1BYO</u>	130	32.6%	89	99	167.5	Plastocyanin <i>Silene pratensis</i>
<u>1AAC</u>	120	32.0%	75	105	162.1	Amicyanin <i>Paracoccus denitrificans</i>
<u>1PCY</u>	140	27.6%	98	99	140.2	Plastocyanin <i>Populus nigra</i>
<u>1BAW</u>	145	33.0%	94	105	131.1	Plastocyanin <i>Phormidium laminosum</i>
<u>1ID2</u>	91	31.4%	70	106	126.1	Amicyanin <i>Paracoccus versutus</i> (<i>Thiobacillus versutus</i>)
<u>1FA4</u>	131	30.1%	103	105	119.9	Plastocyanin <i>Anabaena variabilis</i>

Table S2 - Statistics for the structural-based sequence comparison, as shown in Figure S1, for the type 1 copper proteins: azurin and auracyanin, from different organisms.

PDB code	Smith-Waterman score	%-tage identity	a.a. overlap	Seq. length	z-score	Protein name and Source
<u>2AZA</u>	-	-	-	129	-	Azurin <i>Alcaligenes denitrificans</i>
<u>1ARN</u>	760	86.8%	129	129	923.7	Azurin-II <i>Alcaligenes xylosoxydans</i>
<u>1RKR</u>	623	70.9%	127	129	759.5	Azurin-I <i>Alcaligenes xylosoxydans</i> ncimb 11015
<u>1BEX</u>	563	62.2%	127	128	687.7	Azurin <i>Pseudomonas aeruginosa</i>
<u>1JOI</u>	558	60.8%	125	128	681.7	Azurin <i>Pseudomonas fluorescens</i> A
<u>1NWO</u>	551	59.8%	127	128	673.3	Azurin <i>Pseudomonas putida</i>
<u>1CUO</u>	478	54.3%	127	129	585.8	Azurin iso-2 <i>from Methylomonas j.</i>
<u>1OV8</u>	168	40.7%	86	139	213.8	Auracyanin <i>Chloroflexus aurantiacus</i>

Table S3 - Statistics for the structural-based sequence comparison, as shown in Figure S2, for phytocyanins from different organism.

PDB code	Smith-Waterman score	%-tage identity	a.a. overlap	Seq. length	z-score	Protein name and Source
<u>1JER</u>	-	-	-	110	-	Cucumber stellacyanin
<u>1X9R</u>	330	51.0%	102	105	431.7	Umecyanin from horseradish
<u>1WS8</u>	245	39.4%	104	104	324.2	Mavicyanin from <i>Cucurbita pepo medullosa</i>
<u>2CBP</u>	189	31.1%	103	96	253.8	Cucumber Basic Protein, a blue copper protein
<u>1F56</u>	155	31.2%	96	91	197.2	Spinach plantacyanin

Table S4 – Open reading frames used to predicted secondary structure and were classified according to our methodology.

Organism	Sequence code	Annotated as	Should be annotated as
<i>Octadecabacter antarcticus</i> 307	ZP_05052162.1	Copper binding protein, plastocyanin/azurin family	Pseudoazurin precursor
<i>Hyphomicrobium denitrificans</i>	BAF80137.1	blue copper protein precursor	Pseudoazurin precursor
<i>Actinobacillus pleuropneumoniae</i> serovar 1 str. 4074	ZP_00134822.1	COG3794: Plastocyanin	Pseudoazurin precursor

Figure Legends

Figure S1 – Structural-based sequence alignment of azurins (AZ) and auracyanins (AU) from different organisms, based on SAS in PDBSUM (Laskowski *et al.*, 2005). The residues coordinating the copper ion are marked with an *. Details and statistics of the structures used in the comparison are given in Table S2 in Supplementary Materials. Residue colors for secondary structure definitions: **red = helix, blue = strand, green = turn, black = coil.**

Figure S2 – Structural-based sequence alignment of phycocyanins from different organisms, based on SAS in PDBSUM (Laskowski *et al.*, 2005). The residues coordinating the copper ion are marked with an *. Details and statistics of the structures used in the comparison are given in Table S3 in Supplementary Materials. Residue colors for secondary structure definitions: **red = helix, blue = strand, green = turn, black = coil.**

Figure S3 – Secondary structure predicted using Jpred3 (Cole *et al.*, 2008) of three proteins annotated as belonging to the type 1 copper protein family. In yellow is highlighted the signal peptide region. Residue colors for secondary structure definitions: **red = helix, blue = strand, black = coil.**

Figures

Figure S1

Azurins / Auracyanin

			$\beta 1$	$\beta 2$	$\beta 3$	H1	*	$\beta 4$	H2	H3
<u>2AZA</u>	AZ	-----	AQCEATIESNDAMQYDLKEMVVDK	SCKQFTVHLKHVGMKMA	KSAMGHNWVLTK	EADKEGVATDGMNAGLAQD				
<u>1ARN</u>	AZ	-----	AQCEATVESNDAMQYNVKEIVVDK	SCKQFTMHLKHVGMKMA	KVAMGHNLVLTK	DADKQAVATDGMGAGLAQD				
<u>1RKR</u>	AZ	-----	AECSVDIAGNDGMQFDKKEITVSK	SCKQFTVNLKHPGKLA	KNVMGHNWVLTK	QADMQAVNDGMAAGLDNN				
<u>1BEX</u>	AZ	-----	AECSVDIQGNQMQFNTNAITVDK	SCKQFTVNLKHPGKLA	KNVMGHNWVLST	AADMQGVVTDGMASGLDKD				
<u>1JOI</u>	AZ	-----	AECKVTVDSTDQMSFNTKAIEIDK	SCKTFTVELTHSGSLP	KNVMGHNWVLS	AADMPIASDGMAAGIDKN				
<u>1NWO</u>	AZ	-----	AECKVTVDSTDQMSFNTKAIEIDK	SCKTFTVELTHSGSLP	KNVMGHNWVLS	KEADMPIATDGLSAGIDKQ				
<u>1CUO</u>	AZ	-----	ASCETTVTSGDTMTYSTRISVPA	SCEFTVNFHEKHMPKT	GMGHNWVLAK	SADVGDVAKEGAHAGADNN				
<u>1OV8</u>	AU		ANAPGGSNVVNETPAQTVEVRAAP	DALAFQAQTSLSLP	-A-NTVVRLDFVNQNNL	GVQHNWVLVNGGDDVAAAVNTAAQNNADA				
			$\beta 5$	$\beta 6$	H4	$\beta 7$	*	*	*	$\beta 8$
<u>2AZA</u>	AZ	-YVKAGDT-	RVIAHTKVIIGGESDSVTFD	VSKLTPGEAYAYFC	SFPGHW-	AMMKGTLKLSN				
<u>1ARN</u>	AZ	-YVKAGDT-	RVIAHTKVIIGGESDSVTFD	VSKIAAGENYAYFC	SFPGHW-	AMMKGTLKLSN				
<u>1RKR</u>	AZ	-YVKKDDA-	RVIAHTKVIIGGETDSVTFD	VSKLAAGEDYAYFC	SFPGHF-	ALMKGVCLKLVD				
<u>1BEX</u>	AZ	-YLKPDDS-	RVIAHTKLIIGSEKDSVTFD	VSKLKEGEQYMF	CTFPGHS-	ALMKGTLTLK-				
<u>1JOI</u>	AZ	-YLKEGDT-	RVIAHTKIIGAGEKDSVTFD	VSKLAAAGTDYAFF	CSFPGHI-	SMMKGTVTVK-				
<u>1NWO</u>	AZ	-YLDGDA-	RVIAHTKVIIGAGEKDSVTFD	VSKLAAAGEKYGFF	CSFPGHI-	SMMKGTVTVK-				
<u>1CUO</u>	AZ	-FVTPGDK-	RVIAFTPIIGGEKTSVFK	VSALEKDEAYTYFC	SYPGHF-	SMMRGTCLKLE				
<u>1OV8</u>	AU	LFVPPPDTPNALAWTAMLN	AGESGVSFTRTP	--APG-	TYLYICTFPGHYLAG	MKGTLTVP				

Figure S2

Phytocyanins

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          β1                β2   β3         *   β4
1JER  MQSTVHIVGDNTGWSVPSSPNFYSQWAAAGKTFRVGDSLQFNFANAHNVHEMETKQSFDACNFVN
1X9R  --MEDYDVGGDMEWKRPSDPKFYITWATGKTFRVGDLEFDFAAGMHDVAVV-TKDAFDNCKKEN
1WS8  -MATVHKVGDSTGWTT-LVPYDYAKWASSNKFHVGDSLLFNYNKFHNVLQV-DQEQFKSCNSSS
2CBP  ---AVYVVGSGGWTFNTE-----SWPKGKRFRAGDILLFNYNPSMHNVVVV-NQGGFSTCNTPA
1F56  ---AVYNIGWS-----FNVNGARGKSFRAGDVLVFKYIKGQHNVVAV-NGRGYASCSAPR

          β5                β6   β7   *   *   *   β8
1JER  SDNDVERTSPVIERLDEL-GMHYFVCTVGTHCSNGQKLSINVVAAN-
1X9R  PISHMT-TPPVKIMLNTT-GPQYYICTVGDHCRVGQKLSINVVGA--
1WS8  PAASYT-SGADSIPLKRP-GTFYFLCGIPGHCQLGQKVEIKVDP---
2CBP  GAKVYT-SGRDQIKLPK--GQSYFICNFPGHCQSGMKIAVNAL----
1F56  GARTYS-SGQDRIKLTR--GQNYFICSFPGHCGGMKIAINAK----
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Figure S3

>gi|254438668|ref|ZP_05052162.1| Copper binding protein,
plastocyanin/azurin family [Octadecabacter antarcticus 307]
MRKLLMTTALLIASAVPALAETIEVQMLNENAAGDRMVFSPELIHAEVGDVIKFIATDRSHNAQSVRNAL
PEGQEAFKGRMSQDVEYVVTETGLTAVVCQPHQTMGMVALIVVGDDFSNAQDILDARIRGAGKDKIEALI
EEAQAAQS

>gi|157201179|dbj|BAF80137.1| blue copper protein precursor
[Hyphomicrobium denitrificans]
MKLSRQILFATSITLAAIIVPRFATAAEHIVEMRNKDDAGNTMVFQPGFVKVEAGDTVKFVPTDKSHNAE
SVREVVPEGVAPVKGGFSKEVVFNAEKEGLYVLKCAPHYGMGMVVLVQVGKPVNLDQIKKEYKATGLAKKR
LDGEIAKVQ

>gi|32034684|ref|ZP_00134822.1| COG3794: Plastocyanin [Actinobacillus
pleuropneumoniae serovar 1 str. 4074]
MKKVLATLFAFSGSVFADHEVKMLDHGKDGGMVFEFVKAEVGDTVTFVPTHKGHVQSRRAIPEGAE
KFLSKENEKFTLTLTHEGVVYVCPPHRTMNMMSGIIQVGKPTNLLENATKEIEKIEKRTTENKGRLEFYLD
QVK