

**Supplementary Information for: Proctor *et al.* Zeta-carotene isomerase (Z-ISO) is required for light-independent carotenoid biosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803**

**Table S1. Plasmids used in this study.**

Plasmid name	Details	Source/reference <sup>1</sup>
pCDFDuet <sup>Tm</sup> -1	Has two multiple cloning sites (MCS) for IPTG-inducible coexpression of two genes. AmpR.	Novagen
pAC-ZETAipi	Contains <i>crtE</i> , <i>crtB</i> , and <i>idi</i> genes of <i>Erwinia herbicola</i> (Pantoea agglomerans) Eho10, and <i>pds</i> ( <i>crtP</i> ) gene of <i>Synechococcus</i> PCC 7942 to produce zeta-carotene in <i>E. coli</i> . CmR.	Addgene; Cunningham and Gantt, 2007 [27]
pPD-NFLAG	Genes cloned into NotI/BglII sites are preceded by sequence encoding N-terminal 3xFLAG tag. Integrates into <i>Synechocystis</i> genome at <i>psbAII</i> locus. KanR.	Hollingshead et al. 2012 [32]
pPD-CFLAG	Genes cloned into NdeI/NheI sites are followed by sequence encoding C-terminal 3xFLAG tag. Integrates into <i>Synechocystis</i> genome at <i>psbAII</i> locus. KanR.	Chidgey et al. 2014 [34]
pET21a(+)	Plasmid for IPTG inducible gene expression. AmpR.	Novagen
pMAL-c5x	Allows inducible cytoplasmic expression of maltose-binding protein (MBP) fusions with a Factor Xa cleavage site. AmpR.	New England Biolabs
pMAL-p5x	Allows inducible periplasmic expression of maltose-binding protein (MBP) fusions with a Factor Xa cleavage site. AmpR.	New England Biolabs
pAH592	slr1599 inserted at MCS2 of pCDFDuet <sup>Tm</sup> -1.	This study
pAH595	Maize Z-ISO inserted at MCS2 of pCDFDuet <sup>Tm</sup> -1.	This study
pAH585	<i>Synechocystis</i> <i>zds</i> ( <i>crtQ</i> ; <i>slr0940</i> ) inserted at MCS1 of pCDFDuet <sup>Tm</sup> -1.	This study
pAH587	slr1599 inserted at MCS2 of pAH585.	This study
pAH590	Maize Z-ISO inserted at MCS2 of pAH585.	This study
pAH445	Slr1599 inserted into NotI/BglII sites of pPD-NFLAG	This study
pAH436	<i>Arabidopsis</i> Z-ISO gene (At1g10830) lacking the first 174 bp (the sequence encoding the N-terminal chloroplast transit peptide) and with the sequence encoding a Ser-Ala linker	This study

	followed by the Strep-tag® II added to the 3' end of the gene inserted into NdeI/BglII sites of pPD-NFLAG	
pAH545	Codon optimized His-MBP- <i>ZmZ-ISO</i> construct inserted into pET21a(+)	This study
pAH546	As pAH545 but with the <i>ZmZ-ISO</i> replaced by slr599.	This study
pAH565	As pAH546 but with <i>ynhF</i> inserted upstream of slr1599.	This study
pAH424	slr1599 inserted into pMAL-c5x	This study
pAH707	slr1599 inserted into pMAL-p5x	This study

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<sup>1</sup>See main paper for references.

**Table S2. Primers used in this study.** Restriction sites are shown bold italics, mismatches in QuikChange primers are underlined and homology regions for OLE-PCR are underlined and bold.

Primer	Sequence (5' → 3')	Details
AH1203	AAGCCT <b><i>CATATG</i></b> TTGTTTGATATTTCTTCTCCTTGGC	Primer pair for slr1599 into pCDFDuet MCS2 (NdeI/XhoI)
AH1206	AGCAAT <b><i>CTCGAG</i></b> CTACCAATTAAGTCTGGAGGTC	
AH1207	AAGCCT <b><i>CCATGG</i></b> GGCGTGTTGCAATTGTGGGAG	Primer pair for <i>SynZDS</i> into pCDFDuet MCS1 (NcoI/SalI)
AH1208	AGCAAT <b><i>GTCGACT</i></b> TACTGAGATGCCAGGACTG	
AH912	TAGAACCAACGATTGACCAATTCAC	Primers for generation of slr1599 deletion construct by OLE-PCR (912/913 UsF and 914/915 DsF)
AH913	<b><u>CTGCAATCGGATGCGATTATTGAATA</u></b> AATGGCAAACAACAGAAGGTAGG	
AH914	<b><u>GCATCCCTTAACCTGTTTTTCGTGTG</u></b> TGGGGGTAATGGGTTTCATTGC	
AH915	TTGCATTGTTGCAGGGCTTGG	
AH595	TATTCAATAATCGCATCCGATTGCAG	Primer pair for erythromycin resistance cassette
AH596	CACACGAAAAACAAGTTAAGGGATGC	
AH955	AAGCCT <b><i>GCGGCCGC</i></b> CATTTGATATTTCTTCTCCTTGGC	Primer pair for <i>SynZ</i> -ISO into pPD-NFLAG (NotI/BglII)
AH956	AGCAAT <b><i>AGATCT</i></b> CTACCAATTAAGTCTGGAGGTCATC	
AH957	AAGCCT <b><i>CATATG</i></b> TTTGATATTTCTTCTCCTTGGC	Primer pair for <i>SynZ</i> -ISO into pPD-CFLAG (NdeI/NheI)
AH958	AGCAAT <b><i>GCTAGC</i></b> CAATTAAGTCTGGAGGTCATCAC	
AH198	GGTATATGGATCATAATTGTATGCCCCG	Primer pair to amplify <i>psbAII</i> region from pPD-N/CFLAG
AH199	CTATTCAATCGCTCTAGGCGATCG	
AH47	AAACGCCCTCTGTTTACCCA	Primer pair for screening segregation of the <i>psbAII</i> locus
AH48	TCAACCCGGTACAGAGCTTC	
AH959	TTCTGTTGTTTGCCATCGTT <b><u>GCT</u></b> AGTGGCTTAGCCGCCCTGC	Primer pair for slr1599 H29A mutation
AH960	AGGGCGGCTAAGCCACTAG <b><u>CA</u></b> ACGATGGCAAACAACAGAAGG	
AH961	TCGGTCAAGTAATTTGGT <b><u>C</u></b> CATTGCCACACCCTCTGG	Primer pair for slr1599 C142S mutation
AH962	ACCAGAGGGTGTGGGCAAT <b><u>G</u></b> ACCAAATTACTTGACCGACC	
AH963	AGTAATTTGGTGCATTGCC <b><u>GC</u></b> CACCCTCTGGTTGGGAACC	Primer pair for slr1599 H145A mutation
AH964	TGGTTCCAACAGAGGGT <b><u>GCG</u></b> GCAATGCACCAAATTACTTGACC	
AH965	TTTGAGTCTGGCATGGCG <b><u>CT</u></b> CGCCGTTGGCAACACAAATATG	Primer pair for slr1599 D173A mutation
AH966	TTGTGTTGCCAACGGCG <b><u>AG</u></b> CGCCATGCCAGACTGCAAAGC	
MP487	TGACTTTGGGCGCATATTTGCT <b><u>GCC</u></b> CTTATGCAGG	Primer pair for MBP-ZmZ-ISO H354A
MP488	CCTGCATAAGAGGG <b><u>G</u></b> CAGCGAAATATGCGCCCAAAGTCA	
MP489	CTTTGGGCGCATATTTGCT <b><u>TGG</u></b> CCTTATGCAGGCTC	Primer pair for MBP-ZmZ-ISO H354W
MP490	GAGGCCTGCATAAGAG <b><u>GCA</u></b> AGCGAAATATGCGCCCAAAG	

MP491	CGTAGGGGAACGGGTGTAT <u>GCC</u> GTGCTGTTC	Primer pair for MBP-ZmZ-ISO R170A
MP492	GAACAGCAC <u>GGC</u> CATACACCCGTTCCCCTACG	
MP493	GGGGAACGGGTGTAT <u>IGG</u> GTGCTGTTGCTG	Primer pair for MBP-ZmZ-ISO R170W
MP494	CAGCGAACAGCAC <u>CCA</u> ATACACCCGTTCCCC	
AH923	AAGCCT <b>CATATG</b> TTTGATATTTCTCTCCTTGGC	Primer pair for slr1599 into pMAL-c/p5X (NdeI/Sall)
AH924	AGCAAT <b>GTCGAC</b> CTACCAATTAACCTCTGGAGGTCATC	

**Table S3. Sequences of MBP-Z-ISO fusion genes used in this study.**

Name and sequence	Key
<p><b>MBP-Maize Z-ISO fusion:</b></p> <p>ATG<b>CATCACCACCATCATCATCACCATCATCAT</b>tcttctggtgtagatctgggtaccA  AAATCGAAGAAGGTAAACTGGTAATCTGGATTAAACGGCGATAAAGGCTATAACGGTCT  CGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGGAATTAAAGTCACCGTTGAGCAT  CCGGATAAACTGGAAGAGAAATTCACAGGTTGCGGCAACTGGCGATGGCCCTGACA  TTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGA  AATCACCCTCGACAAAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGGGATGCCGTA  CGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTGATTT  ATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCGCTGGA  TAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCGTAC  TTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACGGCA  AGTAGCAGATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGACCTT  CCTGGTTGACCTGATTAAAAACAAACACATGAATGCAGACACCGATTACTCCATCGCA  GAAGCTGCCCTTTAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGT  CCAACATCGACACCAGCAAAGTGAATTATGGTGTAAACGGTACTGCCGACCTTCAAGGG  TCAACCATCCAAACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAGTCCG  AACAAAGAGCTGGCAAAAGAGTTCTCGAAAACCTATCTGCTGACTGATGAAGGTCTGG  AAGCGGTTAATAAAGACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTACGAGGAAGA  GTTGGCGAAAGATCCACGTATTGCCGCCACCATGGAACGCCAGAAAGGTGAAATC  ATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATGCCGTGCGTACTGCGGTGATCA  ACGCCGCCAGCGGTCGTGACTGTGATGAAGCCCTGAAAGACGCGCAGACTgatta  cgatatcccaggtagacc<b>GAGAACCTGTACTTCCAATCC</b>aatgccatgCGTCCGGCGCGT  GCGGTGGGCGGCGGCATTGAACCGAAAGAAGGCGTGGTGGCGGAAGGCGATGAAAGCG  GCGGCGGCGCGGTGCTGGTGGGCGAAGATAGCGCGGCGTTTGAACGAAAGATCAGAG  CGTGGCGAGCTGGGCGTATTTTTCGGGCATTCTGGGCGCGGTGCTGGTGGCGCTGAAC  GTGCTGTGGATTGATCCGAGCACCGGCGTGGGCACCAAATTTCTGGATGCGGTGGCGA  GCGTGAGCGATAGCCATGAAGTGGTGTGCTGCTGCTGACCATTTATTTTTCGGGTGGT  GCATAGCGGCATGGCGAGCCTGCGTGAAAGCGGCGAAAAAATTGTGGGCGAACGTGTG  TATCGTGTGCTGTTTTCGGGCATTAGCCTGCCGCTGGCGGTGACCACCATTTGTGTATT  TTATTAACCATCGTTATGATGGCACCCAGCTGTGGCAGGTGCAGGGCATTACCGGCAT  TCATGAACCTGCTGTGGTTTAGCAGCTTTATTAGCTTTTTTTTTCTGTATCCGAGCACC  TTTAACCTGCTGGAAGTGGCGGCGGTGGATAAACCGAAACTGCACATGTGGGAAACCG  GCATTATGCGTATTACCCGTCATCCGAGATGGTGGGCCAGGTGATTGGTGCCTGGC  GCATACCCTGTGGATTGGCAATAGCGTGGCGGTGGCGGCGAGCGTGGGCCCTGATTAGC  CATCATCTGTTTGGCGCGTGGAAACGGCGATCGTCTGCTGAGCCGTTATGGCGAAG  CGTTTGAAGTGTGAAAAAACGTACACGCGTATGCCGTTTTCGGCGGATTATTGATGG  CCGTCAGAAACTGCCGAAAGATTATCATAAAGAATTTTTCTGCTGCCGTATGTGGCG  ATTACCATGCTGACCCTGGGCGCGTATTTTTCGCATCCGCTGATGCAGGCGAGCAGCT  ATCAGCTGCCGTGGTAA</p>	<p>Construct encodes following protein:</p> <p>His<sub>10</sub>-tag-linker-  MBP-linker-TEV  cleavage site-linker-  ZmZ-ISO*</p> <p>*starting at Arg49 codon and codon optimized for <i>E. coli</i> B strain using Integrated DNA Technologies tool</p>
<p><b>MBP-Synechocystis Z-ISO fusion version 1:</b></p> <p>ATG<b>CATCACCACCATCATCATCACCATCATCAT</b>tcttctggtgtagatctgggtaccA  AAATCGAAGAAGGTAAACTGGTAATCTGGATTAAACGGCGATAAAGGCTATAACGGTCT  CGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGGAATTAAAGTCACCGTTGAGCAT  CCGGATAAACTGGAAGAGAAATTCACAGGTTGCGGCAACTGGCGATGGCCCTGACA  TTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGA  AATCACCCTCGACAAAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGGGATGCCGTA  CGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTGATTT  ATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCGCTGGA  TAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCGTAC  TTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACGGCA  AGTACGACATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGACCTT</p>	<p>Construct encodes following protein:</p> <p>His<sub>10</sub>-tag-linker-  MBP-linker-TEV  cleavage site-linker-  Slr1599</p>

<p>CCTGGTTGACCTGATTAAAAACAAACACATGAATGCAGACACCGATTACTCCATCGCA  GAAGCTGCCCTTAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGT  CCAACATCGACACCAGCAAAGTGAATTATGGTGTAAACGGTACTGCCGACCTTCAAGGG  TCAACCATCCAAACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAGTCCG  AACAAAGAGCTGGCAAAAGAGTTCCTCGAAAACTATCTGCTGACTGATGAAGGTCTGG  AAGCGGTTAATAAAGACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTACGAGGAAGA  GTTGGCGAAAGATCCACGTATTGCCGCCACCATGGAAAACGCCCAGAAAGGTGAAATC  ATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATGCCGTGCGTACTGCGGTGATCA  ACGCCGCCAGCGGTCGTCAGACTGTGATGAAGCCCTGAAAGACGCGCAGACTgatta  cgatatcccagggtaccGAGAACCTGTACTTCCAATCCaatgccatgAATTGTCTCGCG  CTGTTTGACATCTCTTCCCCGTGGCCATGGTTAACTCCATCTCATGGTATTATTGCTG  CCTATCTGCTGTTGTTTGGCATTGTTCACTCGGGGCTGGCTGCCCTTACGTGCGTGGGG  TGAATCCAAAATCGGTGCCCCGTGGGTATCGTGTAATTTTTGCACTGGTGAGTATCCCA  TTAGCAACTGGTCTTATTATTTATTTCTTCAATCACCGTTATGACGGTTTACAGTTAT  GGCAAGTACAGGGCGTAACAGGGGTAAAGCCTTTAGTATGGATTCTCAGCGCTTTGAG  TTTTTTCTTCTTTTCCCGGCGACGTTTAACTCTGTTAGAGATCGCTGCAATCCAGAAG  CCAGAGATCCATCTTTACGAAACCGGTATTATTCTGTATCTGCCGGCATCCGCAAATGG  TCGGGCAAGTCATCTGGTGCATTGCACATACGCTTTGGCTTGGCACTACGTTTACGCT  GGTAACGTCCCTCGGCCTCATTGCCCATATTGCTTCGCAGTCTGGCACGGGGATCGG  CGGTGGCAACACAAATATGGCGAAGCCTTTCTCGCCGTAAAGCAACGCACGAGCATT  TTCTTTTCAAGCCATTTGGGAAGGCCGTGAGATCCTTGTTTGGCAAGAGTTTATTAA  GCCAGCGTATGTGCGCGTTATGGGTTTATCGCCTTGTTATGGTGGGGGCATCCATGG  TTAATGGTCATGACATCACGGGTAAATTGGTAG</p>	
<p><b>MBP-Synechocystis Z-ISO fusion version 2 (plus YhnF):</b></p> <p>ATGCATCACCACCATCATCATCACCATCATCATtcttctgggtgtagatctgggtaccA  AAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCT  CGCTGAAGTCGGTAAGAAATTTCGAGAAAGATACCGGAATTAAGTCAACGTTGAGCAT  CCGGATAAACTGGAAGAGAAATTCCACAGGTTGCGGCAACTGGCGATGGCCCTGACA  TTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGA  AATCACCCCGGACAAAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGGGATGCCGTA  CGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTGATTT  ATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCGCTGGA  TAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCCTAC  TTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACGGCA  AGTACGACATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGACCTT  CCTGGTTGACCTGATTAAAAACAAACACATGAATGCAGACACCGATTACTCCATCGCA  GAAGCTGCCTTTAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGT  CCAACATCGACACCAGCAAAAGTGAATTATGGTGTAAACGGTACTGCCGACCTTCAAGGG  TCAACCATCCAAACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAGTCCG  AACAAAGAGCTGGCAAAAGAGTTCCTCGAAAACTATCTGCTGACTGATGAAGGTCTGG  AAGCGGTTAATAAAGACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTACGAGGAAGA  GTTGGCGAAAGATCCACGTATTGCCGCCACCATGGAAAACGCCCAGAAAGGTGAAATC  ATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATGCCGTGCGTACTGCGGTGATCA  ACGCCGCCAGCGGTCGTCAGACTGTGATGAAGCCCTGAAAGACGCGCAGACTgatta  cgatatcccagggtaccGAGAACCTGTACTTCCAATCCaatgccatgATGTCCACGGAT  TTAAAAATTTTCACTGGTAACAACGATTATCGTCCTCGGTTTGATCGTAGCCGTGGGTT  TGACTGCCGCGCTGCACAATTGTCTCGCGCTGTTTGACATCTCTTCCCCGTGGCCATG  GTTAACTCCATCTCATGGTATTATTGCTGCCTATCTGCTGTTGTTTGGCATTGTTTAC  TCGGGGCTGGCTCCTTACGTGCGTGGGGTGAATCCAAAATCGGTGCCCGTGGGTATC  GTGTAATTTTTTGCACTGGTGAGTATCCCATAGCAACTGGTCTTATTATTTATTTCTT  CAATCACCGTTATGACGGTTTACAGTTATGGCAAGTACAGGGCGTAACAGGGGTAAAG  CCTTTAGTATGGATTCTCAGCGCTTTGAGTTTTTTCTTCTTTTCCCGGCGACGTTTA  ATCTGTTAGAGATCGCTGCAATCCAGAAGCCAGAGATCCATCTTTACGAAACCGGTAT  TATTCGTATCTGCCGGCATCCGCAAATGGTCGGGCAAGTCATCTGGTGCATTGCACAT  ACGCTTTGGCTTGGCACTACGTTTACGCTGGTAACGTCCCTCGGCCCTATTGCCCATC  ATTGCTTCGCAGTCTGGCACGGGGATCGGCGGTGGCAACACAAATATGGCGAAGCCTT</p>	<p>Construct encodes  following protein:  His<sub>10</sub>-tag-linker-  MBP-linker-TEV  cleavage site-linker-  YhnF-Slr1599</p>

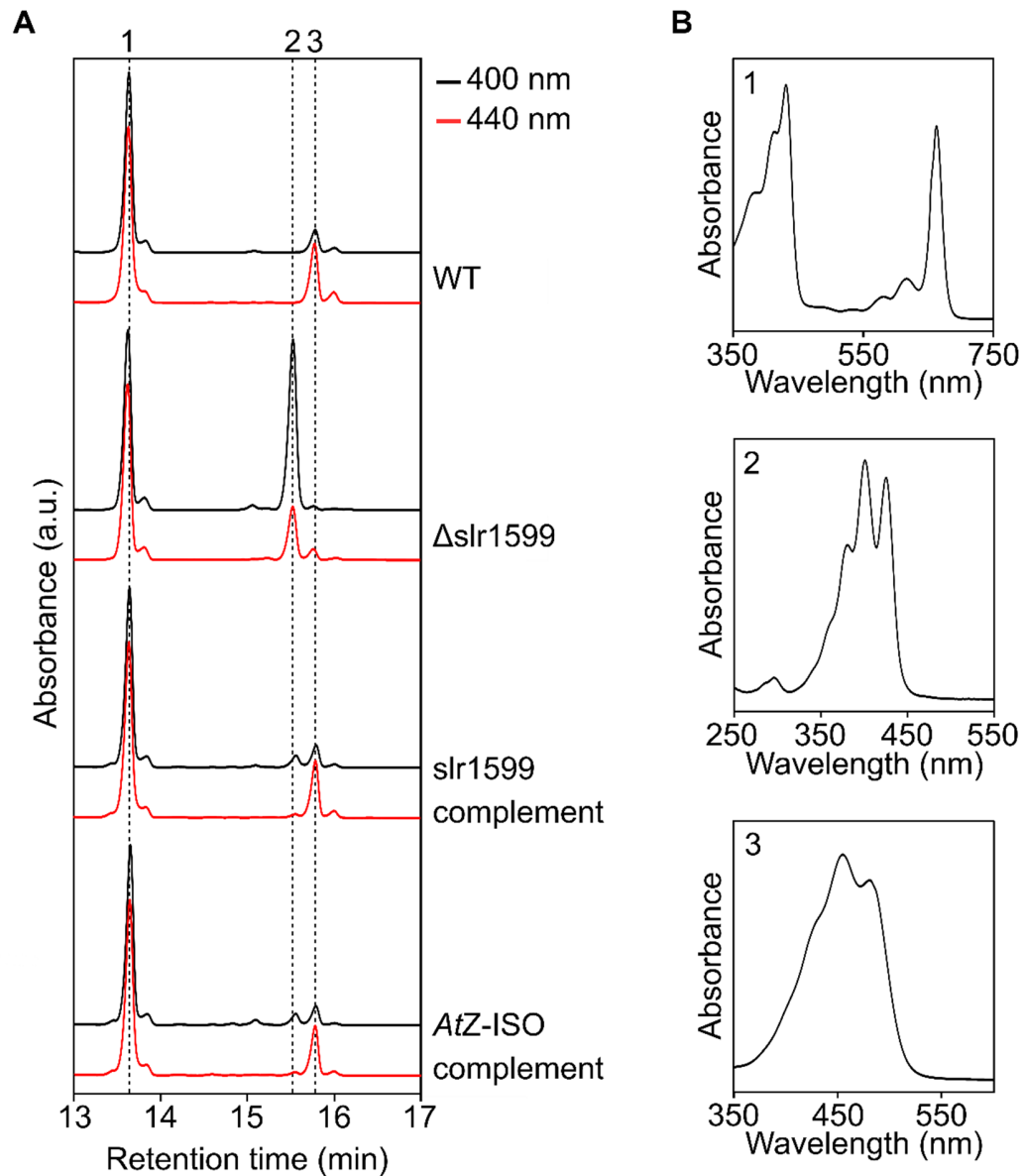
<p>TCTCGCCGTAAAGCAACGCACGAGCATTATTCCTTTTCAAGCCATTTGGGAAGGCCGT CAGATCCTTGTGTTGGCAAGAGTTTATTAAGCCAGCGTATGTCGGCGTTATGGGTTTAA TCGCCCTTGTATGGTGGGGGCATCCATGGTTAATGGTCATGACATCACGGGTAAATTG GTAG</p>	
<p><b>MBP-Synechocystis Z-ISO fusion version 3 (pMAL-c5X):</b></p> <p>ATGAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACG GTCTCGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGGAATTAAAGTCACCGTTGA GCATCCGGATAAACTGGAAGAGAAATCCCACAGGTTGCGGCAACTGGCGATGGCCCT GACATTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGCTCAATCTGGCCTGTTGG CTGAAATCACCCCGGACAAAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGGGATGC CGTACGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTG ATTTATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCGC TGGATAAAGAATGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAGAACC GTACTTCACTGGCCGCTGATTGCTGCTGACGGGGTTATGCGTTCAAGTATGAAAAAC GGCAAGTACGACATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGA CCTTCCTGGTTGACCTGATTAAAAAACAAACACATGAATGCAGACACCGATTACTCCAT CGCAGAAGCTGCCTTTAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCA TGGTCCAACATCGACACCAGCAAAGTGAATTATGGTGTAAACGGTACTGCCGACCTTCA AGGGTCAACCATCCAAACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAG TCCGAACAAAGAGCTGGCAAAAGAGTTCTTCGAAAACCTATCTGCTGACTGATGAAGGT CTGGAAGCGGTTAATAAAGACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTACGAGG AAGAGTTGGCGAAAAGATCCACGTATTGCCGCCACCATGGAACCGCCAGAAAGGTGA AATCATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATGCCGTGCGTACTGCGGTG ATCAACGCCGCCAGCGGTGCTGACATGTCGATGAAGCCCTGAAAGACGCGCAGACTa attcgagctcgaacaacaacaacaataacaataacaacaacctcgggATCGAGGGAAG GatattcacatATGTTTGATATTTCTTCTCCTTGGCCCTGGTTGACCCCCAGCCATGGC ATTATCGCCGCTACCTTCTGTTGTTTGCCATCGTTTCATAGTGGCTTAGCCGCCCTGC GAGCCTGGGGGAAAGCAAAATTGGCGCTAGGGGCTATCGAGTTATATTTGCCCTGGT GAGTATTTCCCTGGCTACGGGATTAATTATTTATTTTCAACCATCGCTATGATGGT CTGCAACTCTGGCAAGTACAGGGAGTAACGGGAGTTAAACCCCTGGTGTGGATTTTGT CTGCCCTATCTTTTCTTCTCTTTTCCCGCCACTTTTAACCTGTTGGAAATTGCCGC TATCCAAAAACCGGAAATCCATCTTTATGAAACGGGCATTATCCGTATTTGTGCCCAT CCTCAAATGGTCCGTCAAGTAATTTGGTGCATTGCCCACACCCCTCTGGTTGGGAACCA CTTTTACTCTGGTTACCAGCTTGGGATTAATTGCCACCATTGCTTTGCAGTCTGGCA TGGCGATCGCCGTTGGCAACACAAATATGGCGAGGCTTTTCTAGCAGTGAAACAAAGA ACTTCCATCATTCCTTTCAAGCGATCTGGGAGGGAAGGCAAAATTCTAGTGTGGCAGG AATTTATCAAACAGCCTATGTGGGGTAATGGGTTTTCATTGCCCTACTATGGTGGGG CCATCCCTGGTTGATGGTGATGACCTCCAGAGTTAATTGGTAG</p>	<p>Construct encodes following protein: MBP-linker-<b>Factor</b> <b>Xa</b> cleavage site- linker-<b>Slr1599</b></p>
<p><b>MBP-Synechocystis Z-ISO fusion version 4 (pMAL-p5X):</b></p> <p>ATGAAAATAAAAACAGGTGCACGCATCCTCGCATTATCCGCATTAAACGACGATGATGT TTTCCGCCCTCGGCTCTCGCCAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGG CGATAAAGGCTATAACGGTCTCGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGGA ATTAAGTCAACCGTTGAGCATCCGGATAAACTGGAAGAGAAATCCCACAGGTTGCGG CAACTGGCGATGGCCCTGACATTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGC TCAATCTGGCCTGTTGGCTGAAATCACCCGGACAAAGCGTTCCAGGACAAGCTGTAT CCGTTTACCTGGGATGCCGTACGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTG TTGAAGCGTTATCGCTGATTTATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTG GGAAGAGATCCCGGCGCTGGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATG TTCAACCTGCAAGAACCGTACTTCACCTGGCCGCTGATTGCTGCTGACGGGGTTATG CGTTCAAGTATGAAAACGGCAAGTACGACATTAAAGACGTGGGCGTGGATAACGCTGG CGCGAAAGCGGTCTGACCTTCTGTTGACCTGATTAAAAACAAACACATGAATGCA GACACCGATTACTCCATCGCAGAAGCTGCCTTTAATAAAGGCGAAACAGCGATGACCA TCAACGGCCCGTGGGCATGGTCCAACATCGACACCAGCAAAGTGAATTATGGTGTAAAC GGTACTGCCGACCTTCAAGGGTCAACCATCCAAACCGTTCTGTTGGCGTGCTGAGCGCA GGTATTAAACGCCGCCAGTCCGAACAAAGAGCTGGCAAAAGAGTTCTTCGAAAACCTATC</p>	<p>Constructs encodes following protein: <b>MalE</b> signal sequence-MBP- linker-<b>Factor Xa</b> cleavage site-linker- <b>Slr1599</b></p>

TGCTGACTGATGAAGGTCTGGAAGCGGTTAATAAAGACAAACCGCTGGGTGCCGTAGC  
GCTGAAGTCTTACGAGGAAGAGTTGGCGAAAGATCCACGTATTGCCGCCACCATGGAA  
AACGCCCAGAAAGGTGAAATCATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATG  
CCGTGCGTACTGCGGTGATCAACGCCGCCAGCGGTCGTCAGACTGTCGATGAAGCCCT  
GAAAGACGCGCAGACTaattcgagctcgaacaacaacaacaataacaataacaacaac  
ctcgggATCGAGGGAAGgatttcacatATGTTTGATATTTCTTCTCCTTGGCCCTGGT  
TGACCCCGAGCCATGGCATTATCGCCGCCTACCTTCTGTTGTTTGCCATCGTTCATAG  
TGGCTTAGCCGCCCTGCGAGCCTGGGGGAAAGCAAATTGGCGCTAGGGGCTATCGA  
GTTATATTTGCCCTGGTGAGTATTTCCCTGGCTACGGGATTAATTATTTATTTTTTCA  
ACCATCGCTATGATGGTCTGCAACTCTGGCAAGTACAGGGAGTAACGGGAGTTAAACC  
CCTGGTGTGGATTTTGTCTGCCCTATCTTTTTTCTTCCTTTTTTCCCGCCACTTTTAAC  
CTGTTGGAAATTGCCGCTATCCAAAACCGGAAATCCATCTTTATGAAACGGGCATTA  
TCCGTATTTGTGCCCATCCTCAAATGGTCGGTCAAGTAATTTGGTGCAATTGCCACAC  
CCTCTGGTTGGGAACCACTTTTACTCTGGTTACCAGCTTGGGATTAATTGCCACCAT  
TGCTTTGCAGTCTGGCATGGCGATCGCCGTTGGCAACACAAATATGGCGAGGCTTTTC  
TAGCAGTGAAACAAAGAACTTCCATCATTCCCTTTCAAGCGATCTGGGAGGGAAGGCA  
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GCCCTACTATGGTGGGGCCATCCCTGGTTGATGGTGATGACCTCCAGAGTTAATTGGT  
AG

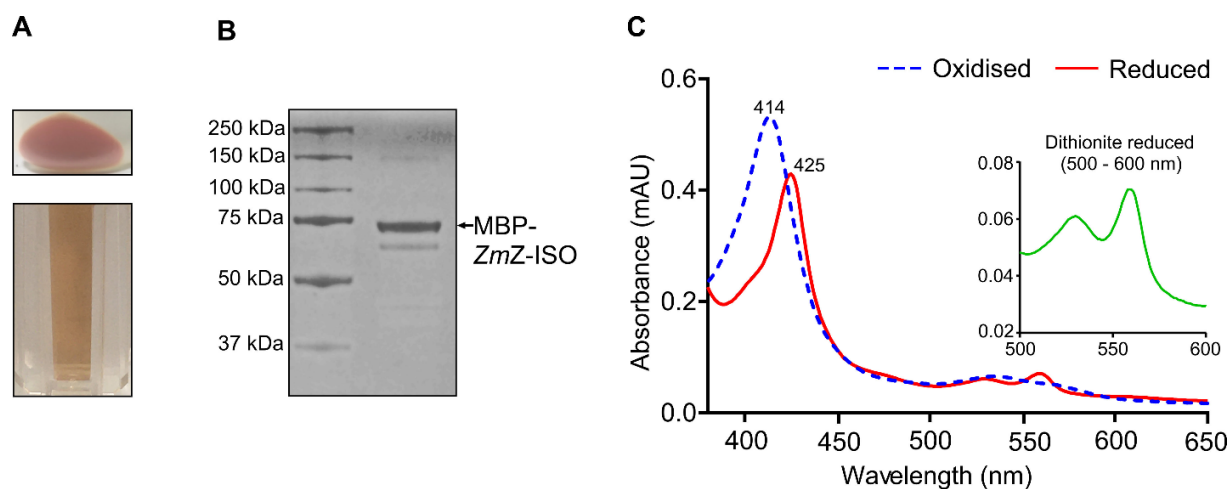


**Table S4. ROSETTA membrane protein scores for abolishment of putative H150 binding site in *Zea mays* Z-ISO.**

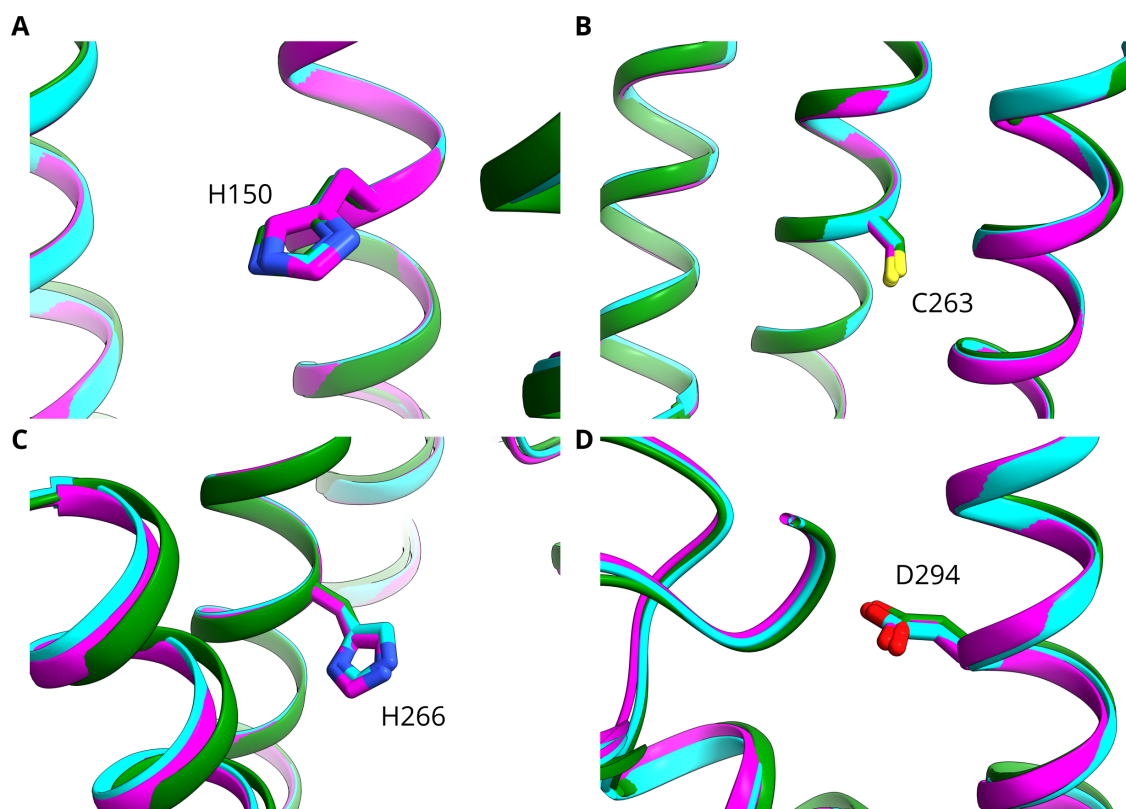
Mutation	ROSETTA score	Mutation	ROSETTA score
WT	-760.705	Arg296Trp	-663.212
Arg170Ala	-762.904	Arg296Phe	-732.487
Arg170Trp	-755.944	Gln255Ala	-766.112
Arg170Phe	-642.435	Gln255Trp	-756.624
Arg296Ala	-758.187	Gln255Phe	-759.713



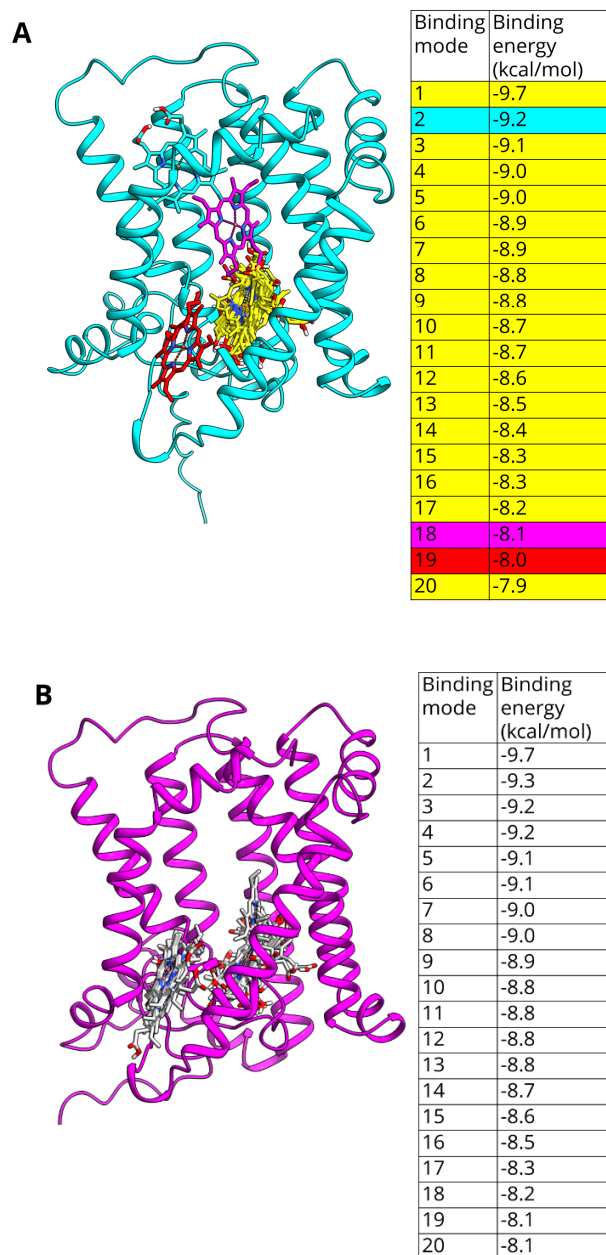
**Figure S1. Complementation of the *Synechocystis*  $\Delta$ slr1599 mutant.** **(A)** Pigments were extracted from WT,  $\Delta$ slr1599 and  $\Delta$ slr1599 with slr1599 or the *Arabidopsis* Z-ISO (AtZ-ISO) genes inserted into the *psbAII* locus (slr1599 complement and AtZ-ISO complement, respectively) and separated by RP-HPLC. Eluted pigments were detected by monitoring absorbance at 400 nm (black trace) and 440 nm (red trace). Peak heights were normalized to the Chl peak (peak 1). Dotted lines and numbers correspond to numbers in **(B)**. **(B)** Eluted pigments in **(A)** were identified by their absorbance spectra. Numbers correspond to equivalent numbered peaks highlighted by dotted lines in **(A)**. Peak 1 = chl *a*; peak 2 = 9,15,9'-tri-*cis*- $\zeta$ -carotene; peak 3 =  $\beta$ -carotene.



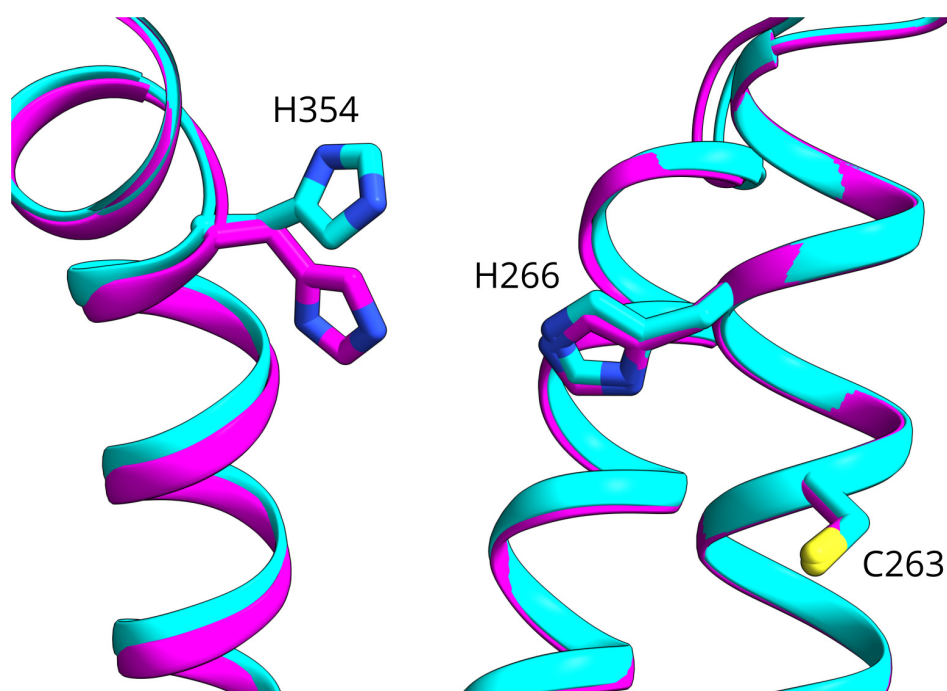
**Figure S2. Purification of the *Zea mays* (Zm) MBP-Z-ISO fusion protein. (A)** Cell pellets (top) and solubilized membrane fractions (bottom) of MBP-ZmZ-ISO heterologously produced in *E. coli*. **(B)** SDS-PAGE analysis of purified MBP-ZmZ-ISO. **(C)** Oxidized and reduced spectra of the MBP-ZmZ-ISO protein with spectral changes in the 500-600 nm region following sodium dithionite reduction highlighted in the inset panel.



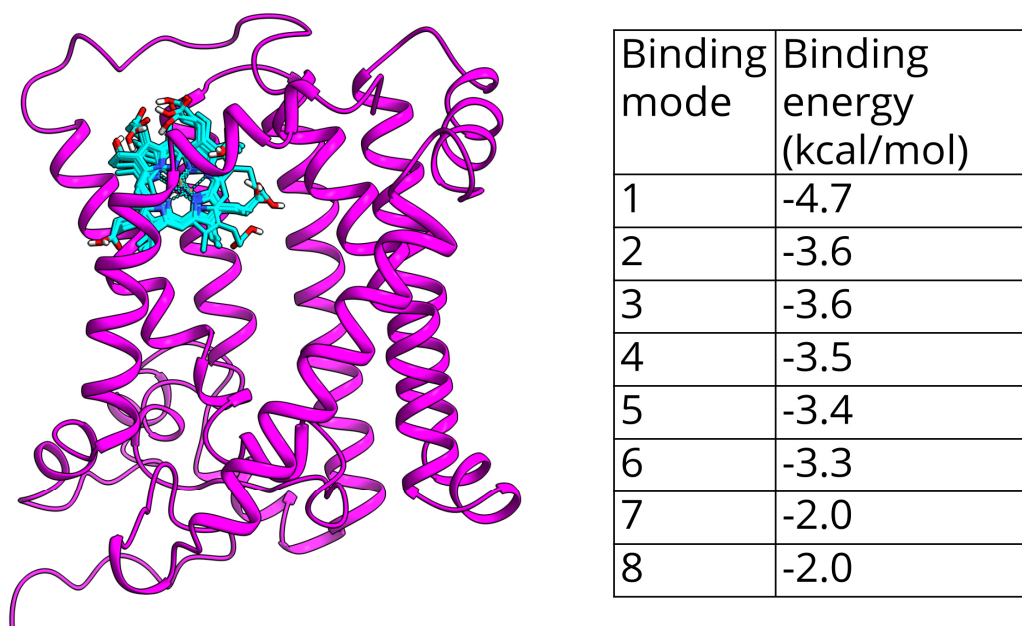
**Figure S3. Orientation of conserved Z-ISO residues with putative catalytic activity.** Z-ISO model structures from *Synechocystis* (green), *Arabidopsis* (cyan) and maize (magenta) are shown, with atomic coordinates of previously-proposed heme binding residues – His 150 (A) and His 266 (C) – and residues with catalytic activity – Cys 263 (B) and Asp 294 (D) – displayed as sticks (oxygen, red; nitrogen, blue; sulfur, yellow).



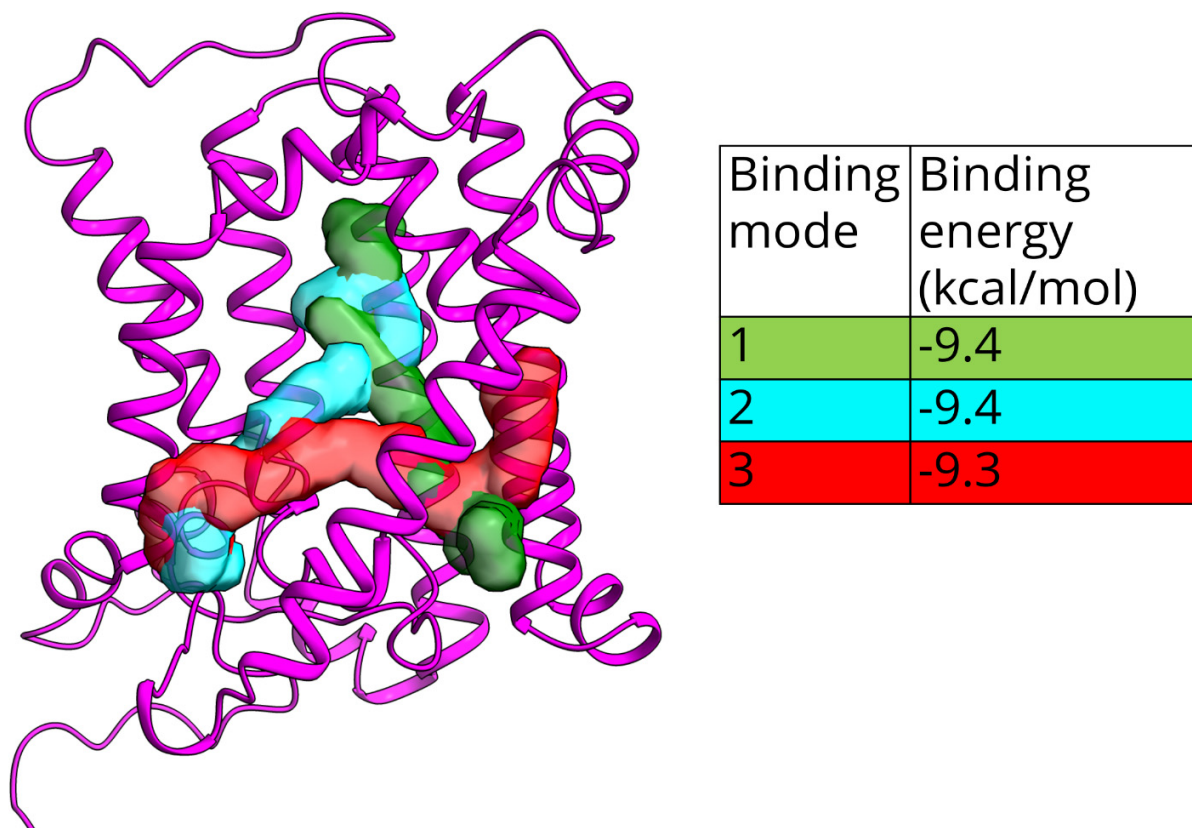
**Figure S4. Heme B docking in *Arabidopsis* (A) and maize (B) Z-ISO using Autodock Vina.** Model protein structures (ribbon) are shown with the heme B cofactor (sticks) positioned in all 20 geometries identified in the docking simulation. The color of the cofactor in (A) corresponds to the color of the cell for the tabulated predicted binding energy estimate displayed in the inset table.



**Figure S5. Alignment of H266 and H354, relative to C263, in maize (magenta) and *Arabidopsis* (cyan) Z-ISO. Key residues displayed as sticks (nitrogen, blue; sulfur, yellow).**

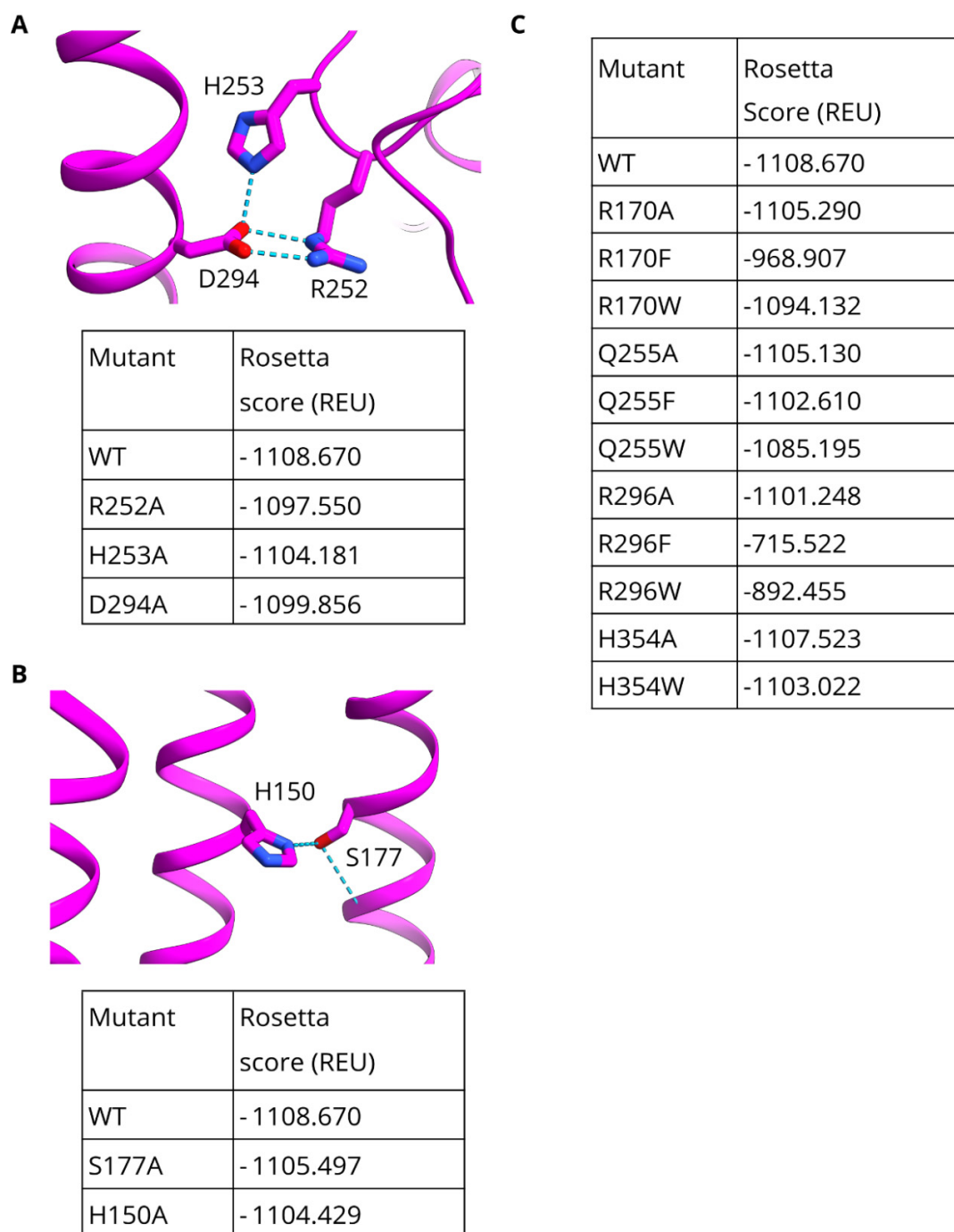


**Figure S6. Heme docking at the H266/H354 site in maize Z-ISO.** Corresponding predicted binding energy values for each heme binding mode are displayed in the table inset (protein, purple; heme-carbon, cyan; oxygen, red; nitrogen, blue; hydrogen, white).



**Figure S7. Docking 9,15,9'-tri-*cis*- $\zeta$ -carotene into the maize Z-ISO-heme B model.** The corresponding predicted binding energy values for each carotenoid binding mode are displayed in the inset table. The color of the carotenoid density corresponds to the color of the cell in the table.





**Figure S8. Using the Rosetta score function to estimate free-energy changes of point mutations.** Predicted hydrogen-bonds between residues proximal to D294 (A) and H150 (B) are shown as dashed lines. Note that only side chain residues are rendered as sticks, backbone hydrogen-bond donors are not visualized. Estimated free-energy changes for point mutations around residues H253 (A, C), H150 (B, C) are displayed in the tables in Rosetta energy units (REU).