Supporting information

The expression and purification of DGD2 - a chloroplast outer membrane associated glycosyltransferase for galactolipid synthesis

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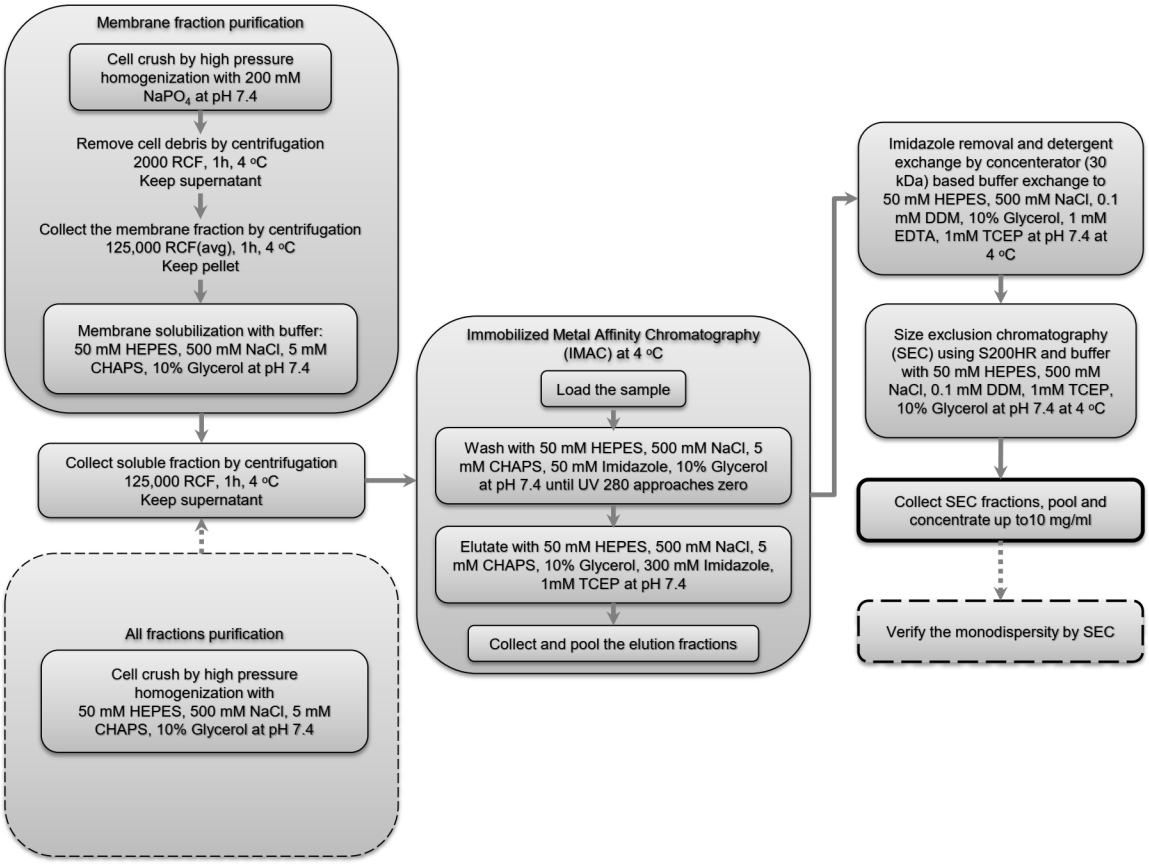


Figure S1. Workflow for DGD2Δ401-473 purification. The target protein can be purified from the membrane fraction exclusively or from all fractions. Dashed line indicates alternative workflow steps. The membrane fraction purification yields sample of better purity.

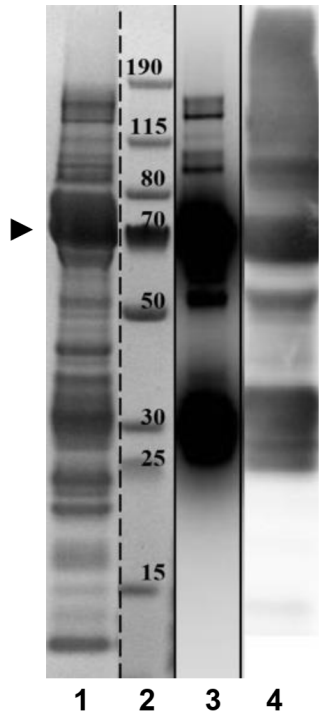
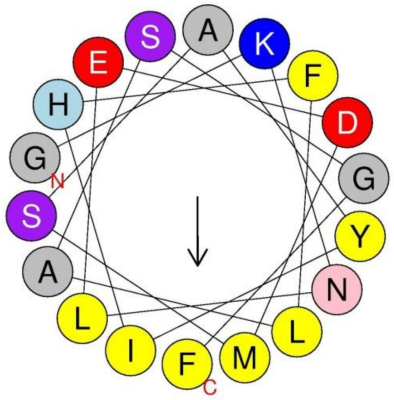


Figure S2. IMAC purification of full length DGD2. Lane 1. Pooled sample after the IMAC step; Lane 2. MW. marker. (Lane 1, 2 are Coomassie stained) Lane 3. In-gel fluorescence image of the lane. Lane 4. Anti-Histag western blot of the lane.

A

$_{401}$SNLSKRSVFASSSISV$_{417}$GKNLEDMSAYIHFLASGF$_{434}$EA
SRTAFGAIPGSLQPDEELCRDLGLSLNTPSPNTRKQD$_{473}$

B



HeliQuest[1] analysis of fragment $_{417}$GKNLEDMSAYIHFLASGF$_{434}$ (18 aa) shows amphiphilic propensity with hydrophobicity <H> = 0.48 and hydrophobic moment <μH> = 0.409.

Figure S3. DGD2 C-terminal 73 residues. (A) The sequence is show with Serine and Threonine underlined. (B) HeliQuest[1] scanning of the sequence identified a potential amphiphilic helix fragment.

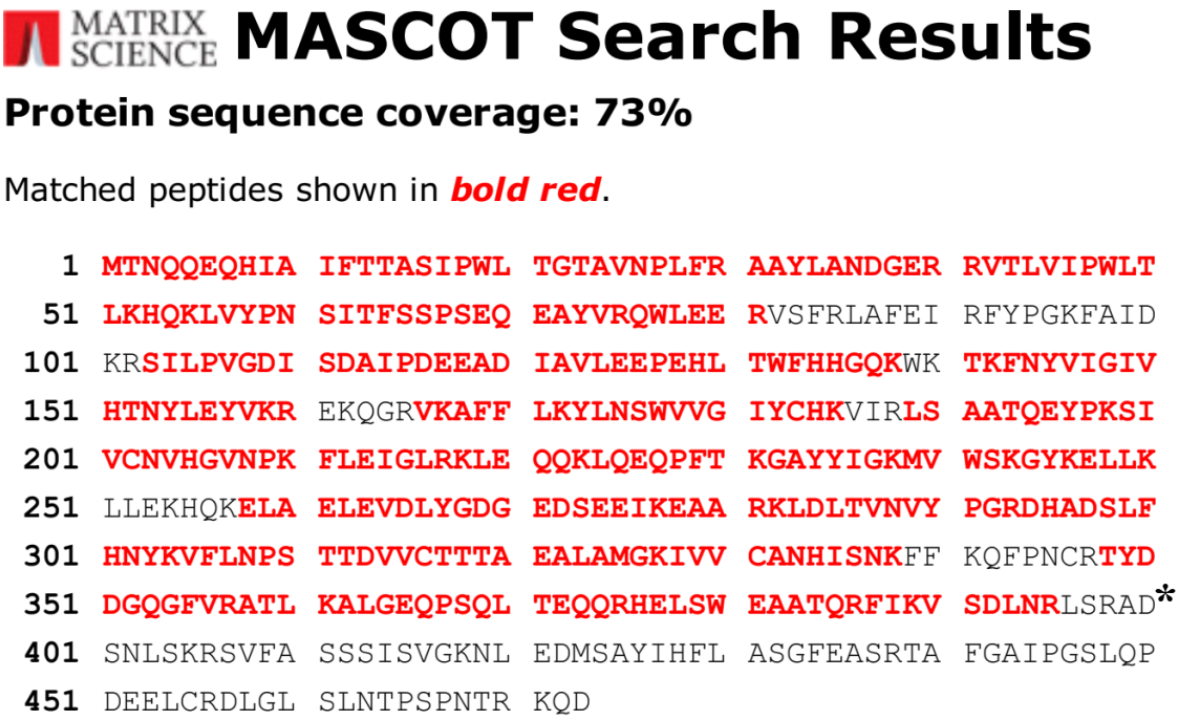


Figure S4. The target recombinant protein band was verified by mass spectrometry based protein sequencing. The protein was digested with trypsin and a MASCOT peptide sequence database search[2] was used to show that the C-terminal part of DGD2 was truncated as designed. An asterix (*) indicates the site of truncation.

Table S1. List of primers.

Primers	Sequence
dgd2-XhoI	5’-GAGACTCGAGATGACGAACCAGCAGGAA-3’
dgd2-BamHI	5’-TATAGGATCCCTTATGCGGAAGTGGCTTATG-3’
dgd2Δ1-178-NdeI	5’-AATACATATGGAAGAACGCGTGTCGTTT-3’
dgd2Δ1-178-XhoI	5'-ATATCTCGAGTTAATCTTGTTTGCGAGTATTCG-3'
dgd2Δ401-473-HindIII	5’-ATATAAGCTTATCGGCCCGTGACAGGCG-3’
dgd2Δ401-473-BamHI	5’-ATATGGATCCATCGGCCCGTGACAGGCG-3’

Enzyme restriction sites are underlined.

Table S2. Optimization of DGD2Δ401-473 purification. List of buffer conditions that have been tested for the purification.

No.	Buffer composition	IMAC yield/purity	SEC monodispersity
1	50 mM NaOAc pH 5.4	-/-	±
2	50 mM Tris-HCl pH 7.4	±/±	±
3	50 mM HEPES	Ref.	Ref.
4	50 mM HEPES 150 mM NaCl	±/-	-
5	50 mM HEPES + 2 mM DTT	±/±	±
6	50 mM HEPES + 1% Triton X-100	±/-	±
7	50 mM HEPES + 0.5% Triton X-100	±/-	±
8	50 mM HEPES + 50 mM Arg&Glu	--/--	n/a
9	50 mM HEPES + 1M Urea	±/-	+

10	50 mM HEPES + 0.1 mM DDM	±/±	++
11	50 mM HEPES + 0.05 mM DDM	±/±	±
12	50 mM HEPES + 0.2 mM DDM	±/±	+
13	50 mM HEPES + 10 mM OG	±/-	+
14	50 mM HEPES + 5 mM CHAPS	±/±	+
15	50 mM HEPES + 5 mM CHAPS exchanged to 0.1 mM DDM during IMAC	±/±	+
16	**50 mM HEPES + 5 mM CHAPS exchange to 0.1 mM DDM during sample concentration**	**±/±**	**+++**
17	50 mM HEPES + 5 mM CHAPS exchange to 0.1 mM DDM during SEC	±/±	±

Unless indicated otherwise, the buffers also include 500 mM NaCl, 10% glycerol and pH is 7.4. TCEP is always added into the buffers to 1 mM after the IMAC purification. The tested buffers are compared to buffer No. 3 as a reference. +/++/+++ indicate result improved, **-/--/---** worsened, ± unchanged, n/a not available. The best condition is highlighted in bold.

Supporting references

1 Gautier, R., Douguet, D., Antonny, B., and Drin, G. (2008) HELIQUEST: a web server to screen sequences with specific alpha-helical properties. *Bioinformatics 24*, 2101–2.

2 Perkins, D. N., Pappin, D. J., Creasy, D. M., and Cottrell, J. S. (1999) Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis 20*, 3551–67.