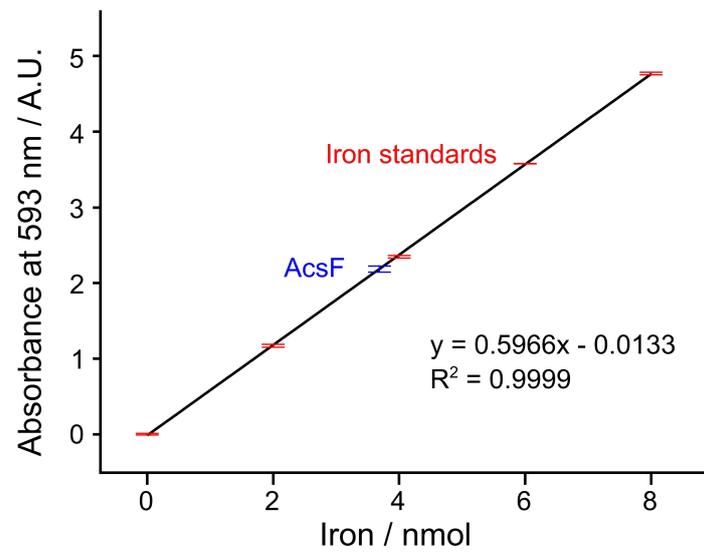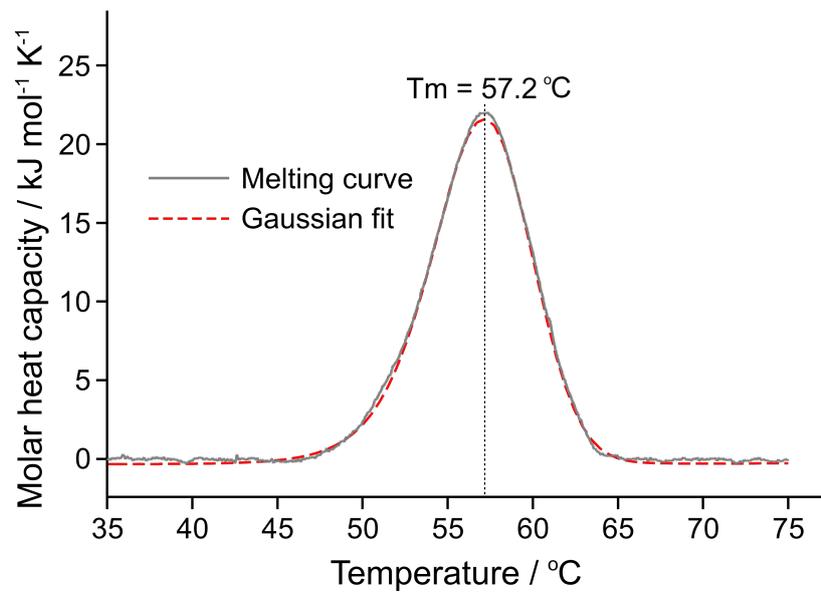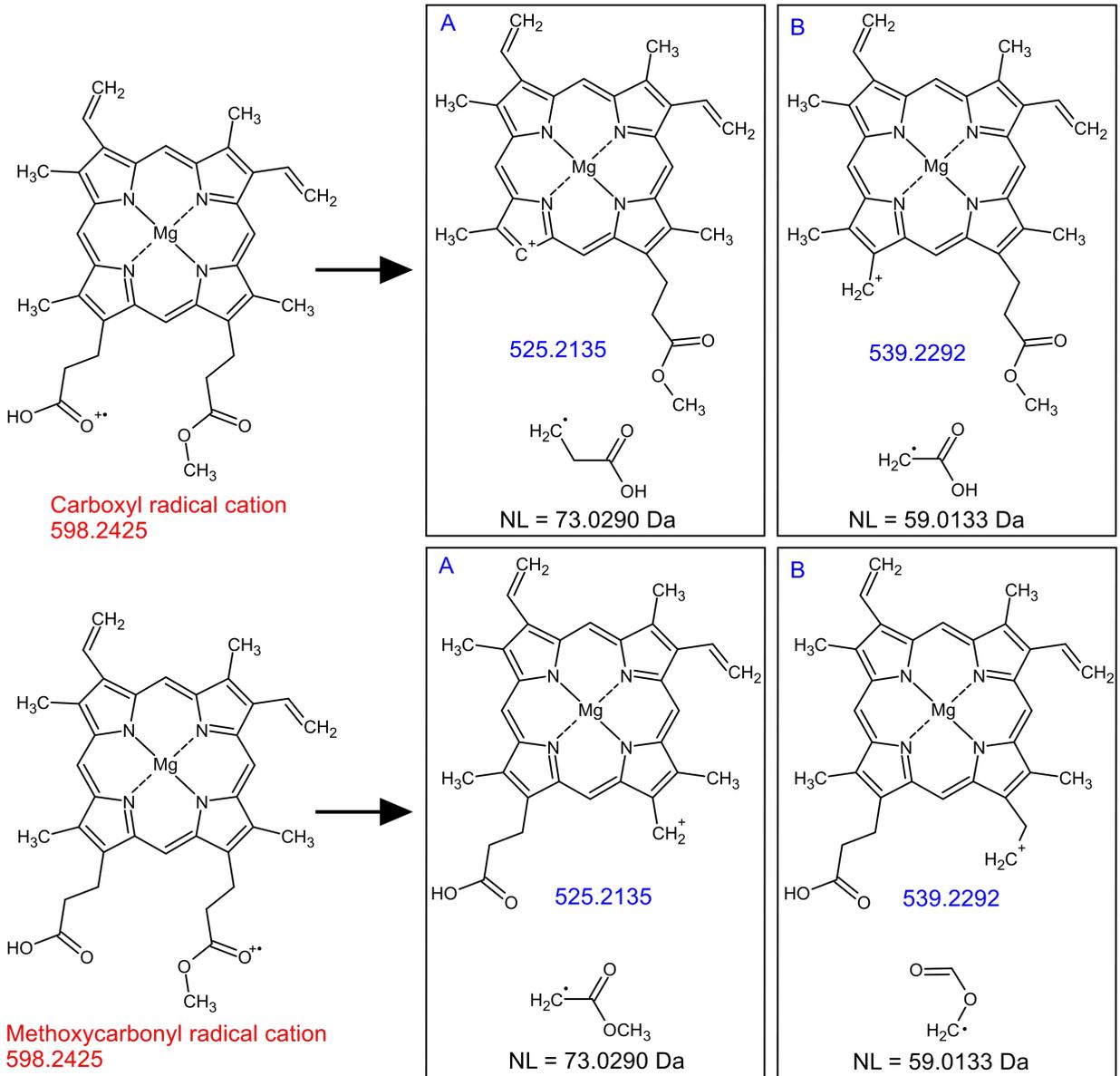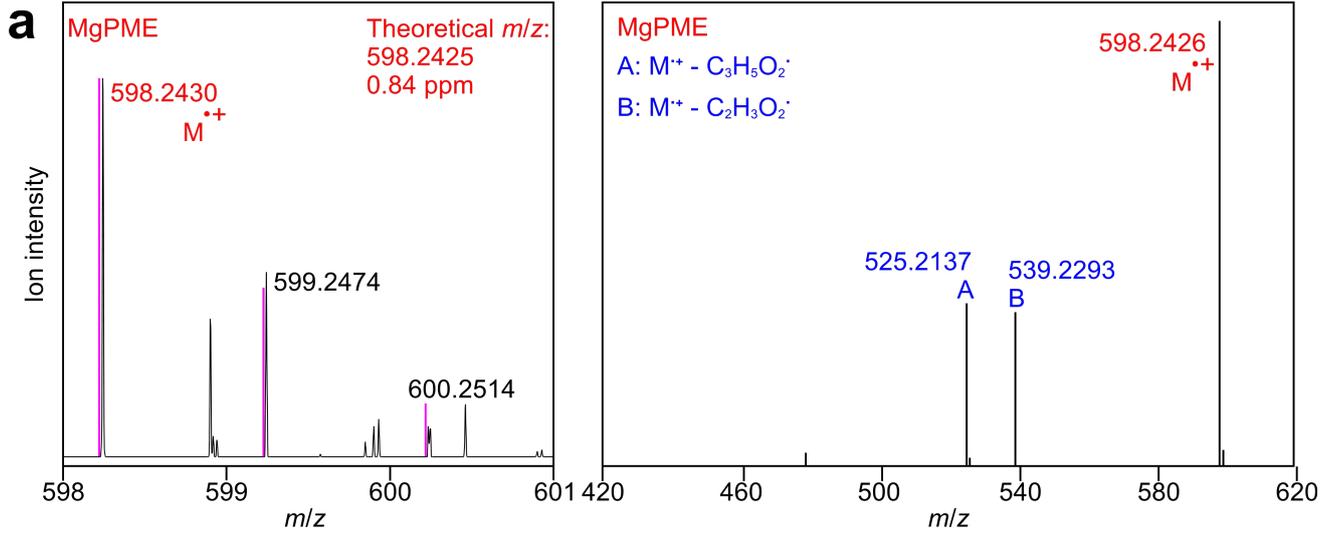## Supplementary Information

Supplementary Figure 1. Determination of the iron content of purified AcsF by iron assay. Error bars indicate the standard deviation from the mean of triplicate experiments. The iron content for 1.569 nmol of AcsF (monomer) was determined to be 3.684 ± 0.066 nmol, producing an iron:AcsF ratio of 2.35 ± 0.04.

Supplementary Figure 2. Melting curve of purified AcsF by differential scanning calorimetry analysis.

a

MgPME
598.2430
M•+

Theoretical *m/z*:
598.2425
0.84 ppm

Ion intensity

599.2474

600.2514

m/z

MgPME
A: M•+ – C₃H₅O₂•
B: M•+ – C₂H₃O₂•

598.2426
M•+

525.2137
A

539.2293
B

m/z

Carboxyl radical cation
598.2425

A
525.2135
NL = 73.0290 Da

B
539.2292
NL = 59.0133 Da

Methoxycarbonyl radical cation
598.2425

A
525.2135
NL = 73.0290 Da

B
539.2292
NL = 59.0133 Da

b

13^1-hydroxy-MgPME

Theoretical m/z:
614.2374
0.49 ppm

Ion intensity

614.2377
M$^{\bullet+}$

615.2414

616.2476

614   615   616   617
m/z

13^1-hydroxy-MgPME
A: M$^{\bullet+}$ - C$_7$H$_{10}$O$_5$
B: M$^{\bullet+}$ - C$_6$H$_9$O$_5^{\bullet}$
C: M$^{\bullet+}$ - C$_5$H$_9$O$_4^{\bullet}$
D: M$^{\bullet+}$ - C$_4$H$_7$O$_4^{\bullet}$

E: M$^{\bullet+}$ - C$_4$H$_5$O$_3^{\bullet}$
F: M$^{\bullet+}$ - C$_3$H$_5$O$_2^{\bullet}$
G: M$^{\bullet+}$ - C$_2$H$_3$O$_2^{\bullet}$

495.2030
D

614.2374
M$^{\bullet+}$

513.2137
E

440.1846*
A

453.1920
B

481.1875
C

541.2086
F

555.2239
G

420   460   500   540   580   620
m/z

Methoxycarbonyl radical cation
614.2374

A
440.1846.

NL = 32.0262 + 142.0266 Da

B
453.1924

NL = 32.0262 + 129.0188 Da

C
481.1873

NL = 32.0262 + 101.0239 Da

D
495.2030

NL = 32.0262 + 87.0082 Da

E
513.2135

NL = 101.0239 Da

b (cont.)

Carboxyl radical cation
614.2374

F
541.2084

NL = 73.0290 Da

G
555.2241

NL = 59.0133 Da

Methoxycarbonyl radical cation
614.2374

F
541.2084

NL = 73.0290 Da

G
555.2241

NL = 59.0133 Da

c

13^1-keto-MgPME

613.2295

612.2222
M$^{•+}$

Theoretical m/z:
612.2218
0.65 ppm

614.2322

Ion intensity

612   613   614   615
m/z

13^1-keto-MgPME

A: M$^{•+}$ - C$_4$H$_4$O$_3$
B: M$^{•+}$ - C$_3$H$_7$O$_3$$^{•}$
C: M$^{•+}$ - C$_3$H$_5$O$_2$$^{•}$
D: M$^{•+}$ - C$_2$H$_3$O$_2$$^{•}$
E: M$^{•+}$ - CH$_4$O

612.2216
M$^{•+}$

580.1959*
E

539.1931
C

521.1826
B

553.2086
D

512.2061*
A

420   460   500   540   580   620
m/z

Methoxycarbonyl radical cation
612.2218

A
512.2058
NL = 100.0160 Da

B
521.1823
NL = 32.0262 + 59.0133 Da

C
539.1928
NL = 73.0290 Da

D
553.2085
NL = 59.0133 Da

E
580.1956
NL = 32.0262 Da

c (cont.)

Carboxyl radical cation
612.2218

C
539.1928
NL = 73.0290 Da

D
553.2085
NL = 59.0133 Da

d

DV PChlide *a*
611.2141
[M + H]+

Theoretical *m/z*:
611.2139
0.49 ppm

610.2065
M•+

612.2170

613.2113

Ion intensity

m/z

DV PChlide *a*

A: [M + H]+ - $C_2H_4O_2$

551.1932
A

611.2092
[M + H]+

m/z

Carboxyl protonated cation
611.2139

A
551.1928

NL = 60.0211 Da

Methoxycarbonyl protonated cation
611.2139

A
551.1928

NL = 60.0211 Da

Supplementary Figure 3. Mass spectral evidence for the hydroxy and keto intermediates in the conversion of MgPME into DV PChlide *a*. Radical cations of MgPME (**a**), 13^1-hydroxy-MgPME (**b**) and 13^1-keto-MgPME (**c**), and the protonated cation of DV PChlide *a* (**d**) are shown in the full-MS spectra in the *top left* panels labelled with their observed *m/z* values and monoisotopic mass accuracies. Also shown in the full-MS spectra are the corresponding ^{13}C isotopomer ions and theoretical relative isotopomer ion intensities calculated from the molecular formulas (magenta lines). The monoisotopic ions were selected for higher-energy C-trap dissociation in a parallel reaction monitoring experiment to genera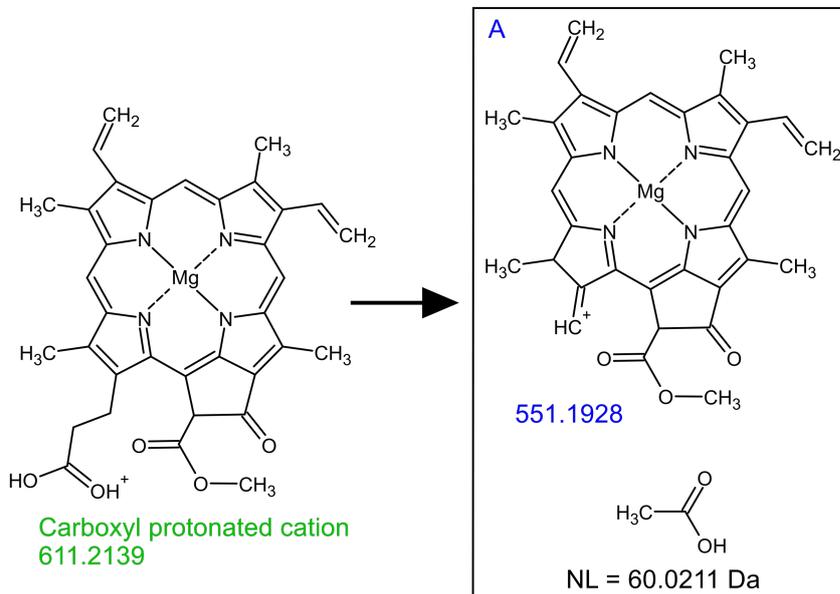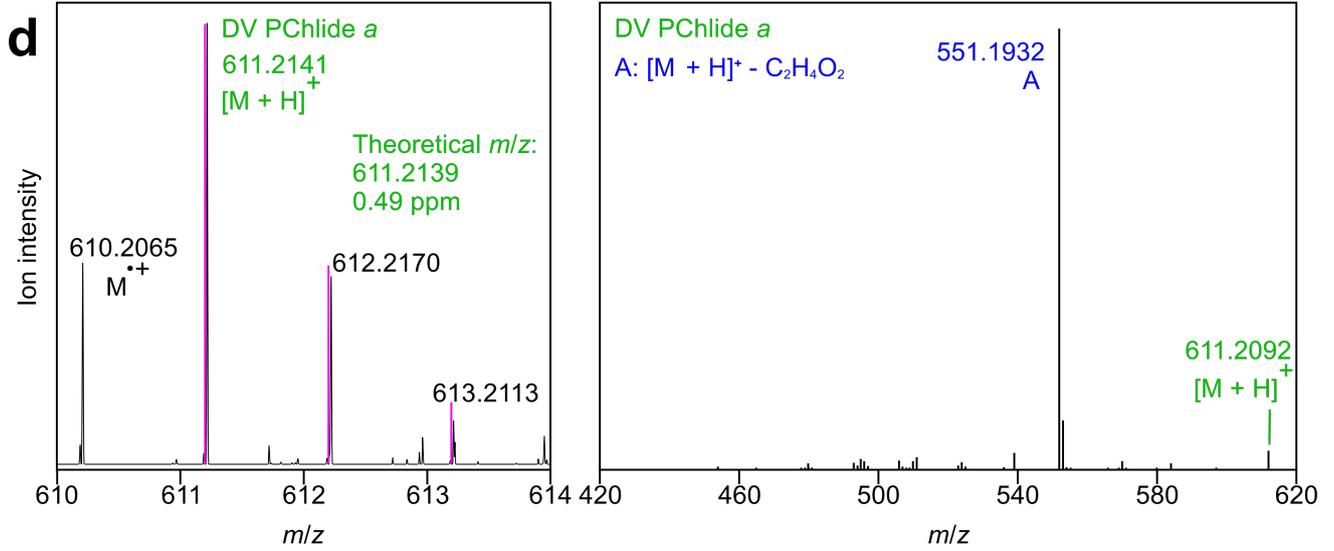te the product ion spectra shown in the *top right* panels, also labelled with their observed *m/z* values. The *bottom* panels show the proposed molecular structures, represented by single canonical forms and labelled with their theoretical *m/z* values, that map to (i) precursor ions identified on the evidence of comparisons between observed and theoretical *m/z* values and isotopomer patterns and (ii) dissociation products of the gas phase neutral loss reactions that are evident as *m/z* species in the product ion spectra. Our identifications of MgPME and its hydroxy and keto derivatives are consistent with the formation of neutral loss species terminating in either carboxyl or methoxycarbonyl groups, depending on which side-chain carries the unpaired electron, with cleavage of successive C-C bonds up to the tetrapyrrole ring to form, in most cases, carbocations (**a**A, **a**B, **b**E–G, **c**C, **c**D). As expected, there was no evidence of dissociation reactions involving the tetrapyrrole ring itself owing to its stability. To account for some of the dissociation pathways exhibited by 13^1-hydroxy-MgPME (**b**) and 13^1-keto-MgPME (**c**) it is probable that an even electron neutral loss of methanol forms an anhydride intermediate that subsequently either undergoes further dissociation (**b**A–D, **c**B) or occurs as a radical cation (**c**E). The dissociation pathways for the protonated cation of DV PChlide *a* that would generate acetic acid and methyl formate, both at 60.0211 Da are shown in **d**. It is proposed, however, that the only significant tetrapyrrole product carbocation detected is from the neutral loss of methyl formate from the methoxycarbonyl radical cation. If the unpaired electron had resided on the carboxyl group, we would have expected to see carbocation products at both 551.1928 and 537.1724 *m/z*, the latter resulting from the neutral loss of propionic acid as seen in the dissociation reactions for MgPME, 13^1-hydroxy-MgPME and 13^1-keto-MgPME (**a**A, **b**F, **c**C). It is therefore apparent that the formation of the isocyclic ring directs preferential protonation of the attached methoxycarbonyl group and, consistent with its expected stability, does not dissociate under the conditions used in this analysis thus restricting the potential number of neutral losses to one. All *m/z* assignments, in terms of odd and even integer masses follow the Nitrogen Rule, i.e. molecules containing an even number of N atoms (like tetrapyrroles) form even electron ions with odd integer masses and vice versa. The majority of tetrapyrrole product ions detected here are even electron carbocations with odd integer masses. The three occurrences of odd electron radical cation products with even integer masses are marked with an asterisk. The spectral peaks at 613.2295 and 614.2322 *m/z* in **c** are more intense than would be expected for the 13^1-keto-MgPME $^{13}C_1$ and $^{13}C_2$ isotopomer ions (theoretical intensities are shown by magenta lines) owing to the presence of merged unidentified coincident ions.
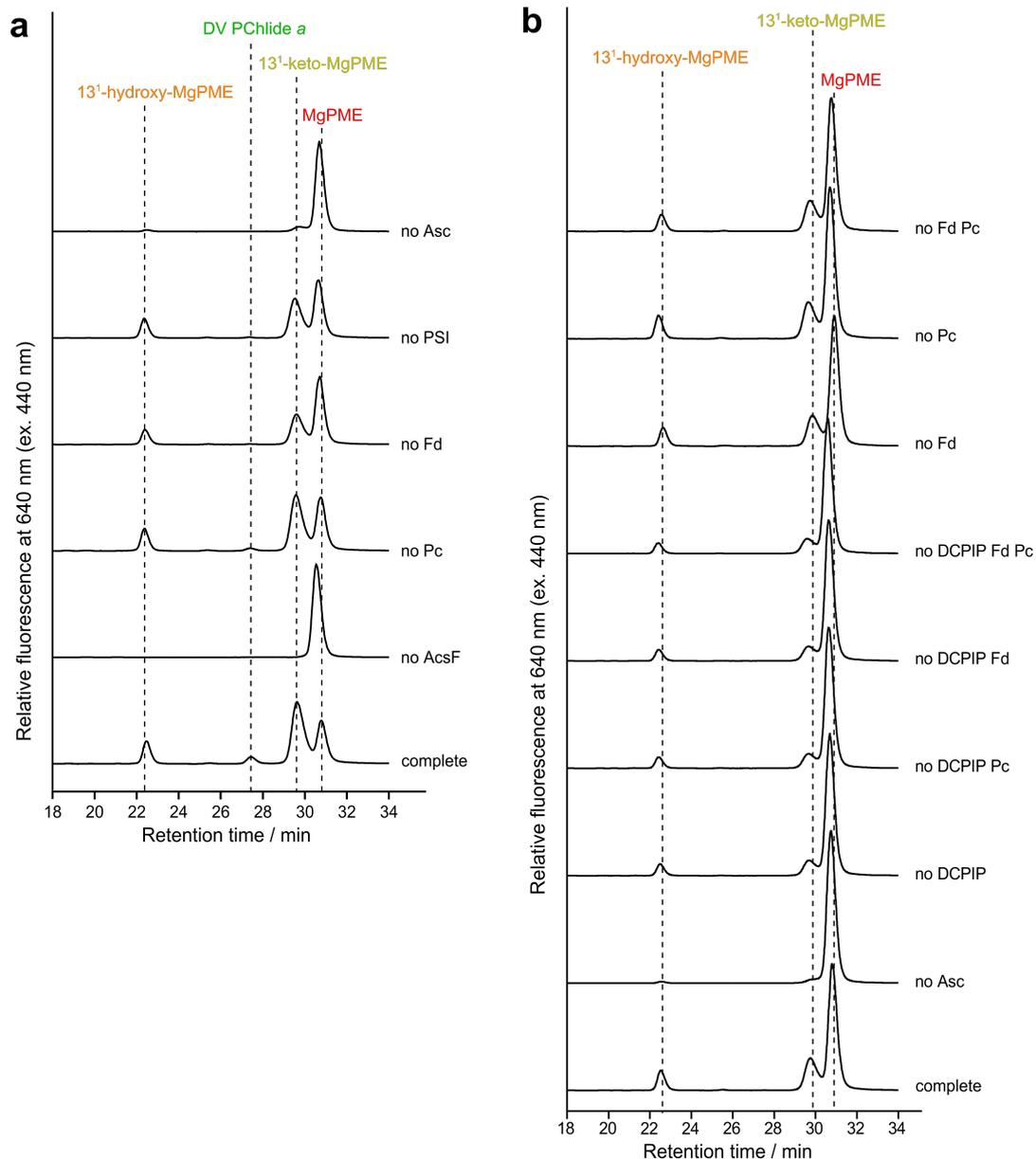
Supplementary Figure 4. HPLC analysis of pigment extracts from coupled PSI-cyclase assay controls. Pigment extracts from assays were analysed by HPLC with pigment elution monitored by fluorescence at 640 nm with excitation at 440 nm. Pigment species were identified by retention times and fluorescence spectra (as in Fig. 4). **a**, Cofactor requirements for cyclase activity in the coupled PSI-cyclase assays. A complete assay contained 2 µM AcsF, 0.04 mg mL^{-1} spinach Fd, 14 µM MgPME, spinach PSI containing 6 µM Chl *a*, 20 µM spinach Pc, 2 mM Asc, 60 µM DCPIP and 0.29 mg mL^{-1} catalase. Assays were incubated under red light illumination for 30 min. **b**, Partial cyclase activity in the absence of PSI. A complete assay contained 2 µM AcsF, 0.06 mg mL^{-1} spinach Fd, 14 µM MgPME, 20 µM spinach Pc, 3 mM Asc, 60 µM DCPIP and 0.29 mg mL^{-1} catalase. Assays were incubated in the dark for 30 min.

Supplementary Table 1. Nucleotide sequences of synthesised genes used in this study*.

Gene	Sequence (5'-3')
petF	ATGGCTACTTTCAAAGTTACCTTGATCAACGAGGCAGAAGGGACTAAGCATGAAATCGAGGTTCCAGAC GACGAGTACATCCTTGATGCGGCAGAGGAACAGGGCTATGATTTGCCCTTTTCATGCCGCGCAGGTGCA TGTTCCACATGCGCCGGTAAGTTGGTATCGGGCACTGTAGACCAATCGGATCAAAGTTTCCTGGACGAC GACCAAATTGAAGCCGGCTACGTCCTGACTTGTGTCGCTTATCCGACATCAGATGTAGTTATCCAAACG CACAAAGAGGAAGATTTGTATTAA
petH	ATG<u>GGT</u>ACCCAGGCTAAAGCCAAGCACGCTGACGTTCCTGTCAATCTTTACCGCCCAAATGCCCCCTTC ATCGGCAAGGTGATCTCAAACGAACCCTTAGTGAAGGAGGGAGGCATTGGAATTGTTCAGCACATTAAA TTTGATCTTACAGGCGGAAACTTAAAGTACATCGAGGGGCAGTCAATCGGCATTATTCCGCCCGGCGTA GACAAAAATGGCAAGCCTGAAAAATTACGTTTGTATTCTATTGCAAGCACTCGTCACGGTGATGATGTG GATGATAAAACTATTTCCCTGTGCGTCCGCCAACTGGAGTATAAGCATCCCGAGTCAGGAGAAACGGTG TACGGAGTCTGCTCTACGTACCTTACTCACATTGAGCCGGGATCTGAAGTTAAAATTACCGGCCCTGTG GGGAAGGAAATGCTGTTGCCAGATGATCCAGAGGCTAACGTTATCATGTTGGCAACCGGGACCGGTATC GCCCCCATGCGTACCTACTTGTGGCGTATGTTTAAGGACGCAGAACGTGCCGCTAACCCGGAGTATCAA TTCAAGGGGTTCAGTTGGCTTGTTTTCGGAGTCCCAACGACTCCGAATATCTTATACAAGGAAGAGTTG GAGGAGATCCAACAAAGTACCCTGATAACTTTCGTCTTACCTACGCTATCTCCCGTGAGCAGAAAAAT CCCCAGGGCGGTCGTATGTATATTCAGGACCGCGTGGCAGAACACGCGGACGAGTTATGGCAGCTGATT AAAAATCAAAAGACACACACGTATATTTGTGGCCTTCGCGGAATGGAAGAGGGAATTGACGCTGCTCTG TCGGCTGCTGCTGCCAAGGAGGGTGTTACTTGGTCGGACTACCAGAAGGATCTTAAGAAGGCCGGACGC TGGCATGTAGAGACTTACTAA

*Genes are codon optimised for expression in *E. coli*. The *petF* gene encodes the *Anabaena* Fd. The *petH* gene encodes the *Anabaena* FNR lacking the N-terminal 136 aa. For cloning reason, a GGT sequence (underlined) was added to the *petH* gene, which encodes a Gly at aa position #2.