SUPPLEMENTAL DATA

Construction of genes encoding GFP-tagged silaffin fragments and pentalysine clusters - The genomic DNA regions corresponding to the sequences of the Sil3-derived C-terminal segments SP, T1-T2 were amplified by PCR using the oligonucleotide primers listed in Table S1, digested with KpnI, and introduced into the EcoRV and KpnI sites of the vector pTpfcp/ctGFP. For Sil3-derived N-terminal segments and internal Sil3 truncations T4-T8 and T10-T12 the corresponding DNA region was amplified by PCR from gDNA using the oligonucleotide primers listed in Table S1, digested with KpnI and introduced into the KpnI site of the plasmid harboring the SP-GFP encoding gene, which resulted in a GFP fusion between the Sil3 signal peptide and the Sil3 truncation that was lacking a signal peptide. The gene segments corresponding to $T7^{scr}$, $T7^{K\rightarrow R+}$ and $T6^{K\rightarrow R+}$ were synthesized by Genscript (Piscataway, NJ, USA) and ligated into the KpnI site of the plasmid harboring the SP-GFP encoding gene. For PLC3ct, PLC1, PLCart, and mutations to Sil3 fragments T6 and T7 two complementary oligonucleotides were annealed (Table S3), and then introduced into the KpnI site of the plasmid harboring the SP-GFP encoding gene. For PLC3ct, and PLCart the two oligonucleotides were designed to have KpnI compatible 3'-ends once annealed. For the other mutants of T6 and T7 the method of oligonucleotide annealing followed by primer extension with Pfx DNA polymerase (Invitrogen) was used. The resulting DNA fragments were introduced into pJet1.2 (Fermentas) for sequencing and then introduced into the KpnI site of the plasmid harboring the SP-GFP encoding gene.

For construction of pTpfcpPLC1 the Sil1 signal peptide of Sil1 was amplified using the sense primer 5'-ACCAAAATGAAAGTTACCACGTCAATCATC-3' and the antisense primer 5'-GATCGGTACCTGC AGCGCCGCAAGAAGC-3' (KpnI site underlined). The resulting 70 bp DNA fragment was digested with KpnI and cloned into the EcoRV and KpnI sites of pTpfcp/ctGFP resulting in pTpfcpSil1SP/GFP. The Sil1 PLC1 sequence was amplified using the sense primer 5'-GATCGGTAC CAAGGCAACCAAGACCCTCAAG-3' and the antisense primer 5'-GATCGGTACCCTTTCCGGA TTTGCTAACCTT-3' (KpnI sites underlined). The resulting 59 bp DNA fragment was digested with KpnI and cloned into the KpnI site of pTpfcpSil1SP/GFP.

Construction of the expression vector for nitrate-inducible expression of *T8-GFP* has been described previously (1).

Table S1. Oligonucleotide primers.

Name	**Primer Sequence (5' to 3')** (KpnI site underlined)
SP, T1, T2, T3-for	ACCAAAATGAAGACTTCTGCCATTG
SP-rev	ATTCGGTACCGGCAGCAGTGGTGGCGAGAAC
T1-rev	ATTCGGTACCAAGAGTTCTCAATCGGCG
T3-rev	ATTCGGTACCTGCAGGGCCAGCAACATC
T2-rev	ATTCGGTACCGACGTCCTCGGCCTCGGCC
T4, T5-rev	ATTCGGTACCAGCGCTCATGGAGTGGACC
T4-for	GAATGGTACCACTGAGATGTCCATGGCAAAG
T5-for	GAATGGTACCATGCCCTCTTCGAAGGCTGC
T9-for	GGTACCTCCGGGAGTCTCTCCATGCTC
T9-rev	GGTACCATGCCAAAAGCTGAGAAGGTC
T6, T7, T8-for	GATCGGTACCCTTCCCACTCTTTCCCTTGAA
T8-rev	GATCGGTACCAAATCGAAGCAAGGAAAGACC
T7-rev	GATCGGTACCAAGGCCTCAAAGGAGTCTAG
T6-rev	GATCGGTACCGAGTCTAGCATGCCCTCTTC
T10-for	GATCGGTACCGACGCAGCTGCCGTAGATG
T10-rev	GATCGGTACCCTTGTCACTTTTAGCGAAGGGC
T11, T12-for	GATCGGTACCATGCCTGATGAGGCCGGTG
T11-rev	GATCGGTACCCTTGTGTGACTTGGCCATGC
T12-rev	GATCGGTACCCTTGGAAGCTTTGGCATCTAC

Table S2. Sil3-derived gene segments that were purchased from a commercial vendor (Genscript).

Name	DNA Sequence (5' to 3')	Peptide Sequence
$T7^{scr}$	GGTACCAAGAAGAAAAAGTTCATGTCAGCCAAGAAGAAAA AGGAGGCTTCTAAGAAAAAGATCCCCGGAAGCAAGAAAAA GGGGAGTTCGGCCTCTAAGAAAAAGGGTAC	GTKKKKFMSAKKKKEASKKK IPGSKKKGSSASKKKGT
$T7^{K\rightarrow R+}$	GGTACCAGGAGACGTGCCTCACGTGAGTCTAGCATGCCCT CTTCGCGAAGAAGGGCTGCCAGACGTAGGATCTTCCGAGG AAGACGTAGGAGTGGGCGTCGAAGGGGTACC	GTRRRARESSMPSSRRRAAR RIFRGRRRSGRRRGT
$T6^{K\rightarrow R+}$	GGTACCGAGTCTAGCATGCCCTCTTCGCGAAGAAGGGCTG CCAGACGTAGGATCTTCCGAGGAAGACGTAGGAGTGGGCG TCGAAGGGGTACC	GTESSMPSSRRRAARRRIFR GRRRSGRRRGT

Table S3. DNA oligonucleotide sequences that were used to synthesize Sil3-derived gene segments by annealing.

Name	DNA Sequences of oligonucleotides (5' to 3')	Protein Sequence
PLC3ct	Sense: GGTACCAAGGCTGCCAAGATCTTCAAGGGAAAGAGTGGGAA GGGTAC Antisense: GGTACCTTCCCACTCTTTCCCTTGAAGATCTTGGCAGCCTTGGTAC	GTKAAKIFKGKSG KGT
PLCart	Sense: GAATGGTACCAAGAGCAGCAAGTCCAGCAAA Antisense: AGTCGGTACCCTTACTTGACTTGGAACTTTTGCTGGACTT	GTKSSKSSKSSKS SKGT
$T6^{S\rightarrow A}$	Sense: ATGGTACCGAGGCTGCCATGCCCGCTGCGAAGGCTGCCAAGATC Antisense: GCGGTACCCTTCCCAGCCTTTCCCTTGAAGATCTTGGCAGC	GTEAAMPAAKAAK IFKGKAGKGT
$T6^{S\rightarrow E}$	Sense: ATGGTACCGAGGAAGAGATGCCCGAAGAGAAGGCTGCCAAGATC Antisense: GCGGTACCCTTCCCTTCCTTTCCCTTGAAGATCTTGGCAGC	GTEEEMPEEKAAK IFKGKEGKGT

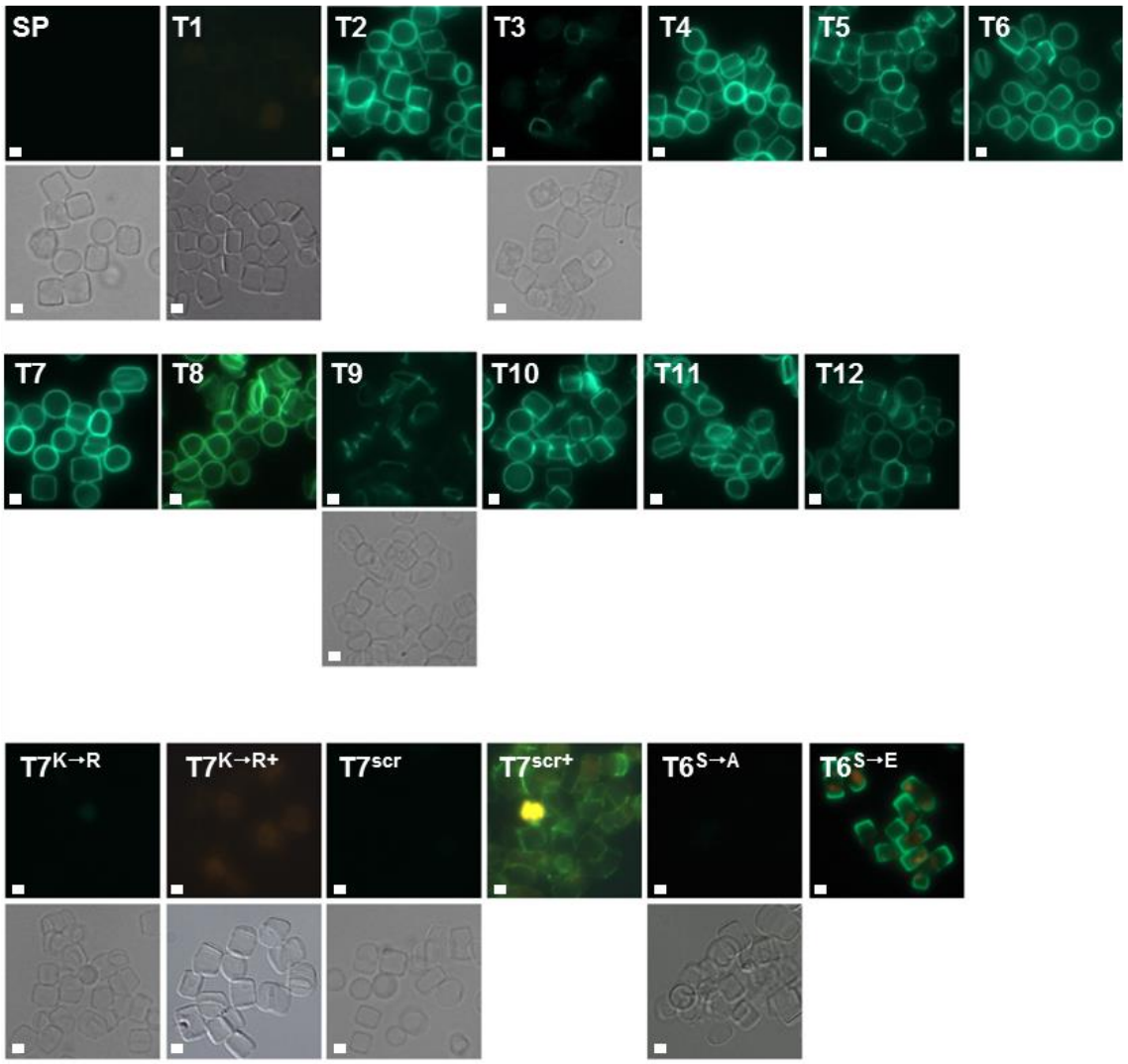

FIGURE S1. Analysis of biosilica incorporation of Sil3-derived GFP fusion proteins. Epifluorescence microscopy images ("F") are shown of isolated biosilica from multiple cells of each transformant strain expressing the indicated Sil3-derived GFP fusion protein. For strains exhibiting no or very weak biosilica-associated GFP fluorescence, the corresponding bright field images ("BF") of the isolated biosilica are shown. White bars: 2 µm.

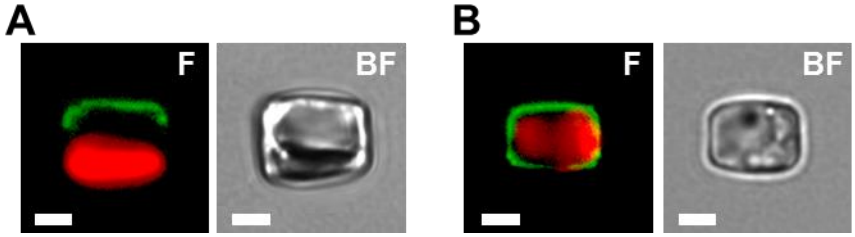

FIGURE S2. Expression of T8-GFP in *T. pseudonana* under control of the nitrate reductase promoter/terminator cassette. Cells are shown in girdle view. Green color is indicative of Sil3-GFP, and red color is caused by chloroplast autofluorescence. White bars: 2 µm. F: Fluorescence, BF: bright field. A. Cell shortly after cell division and separation. B. Cell after progression through 2 cell cycles.

REFERENCES

1. V. C. Sheppard, A. Scheffel, N. Poulsen, N. Kröger (2012) Live Diatom Silica Immobilization of Multimeric and Redox-active Enzymes. *Appl. Environ. Microbiol.* **78**, 211-218