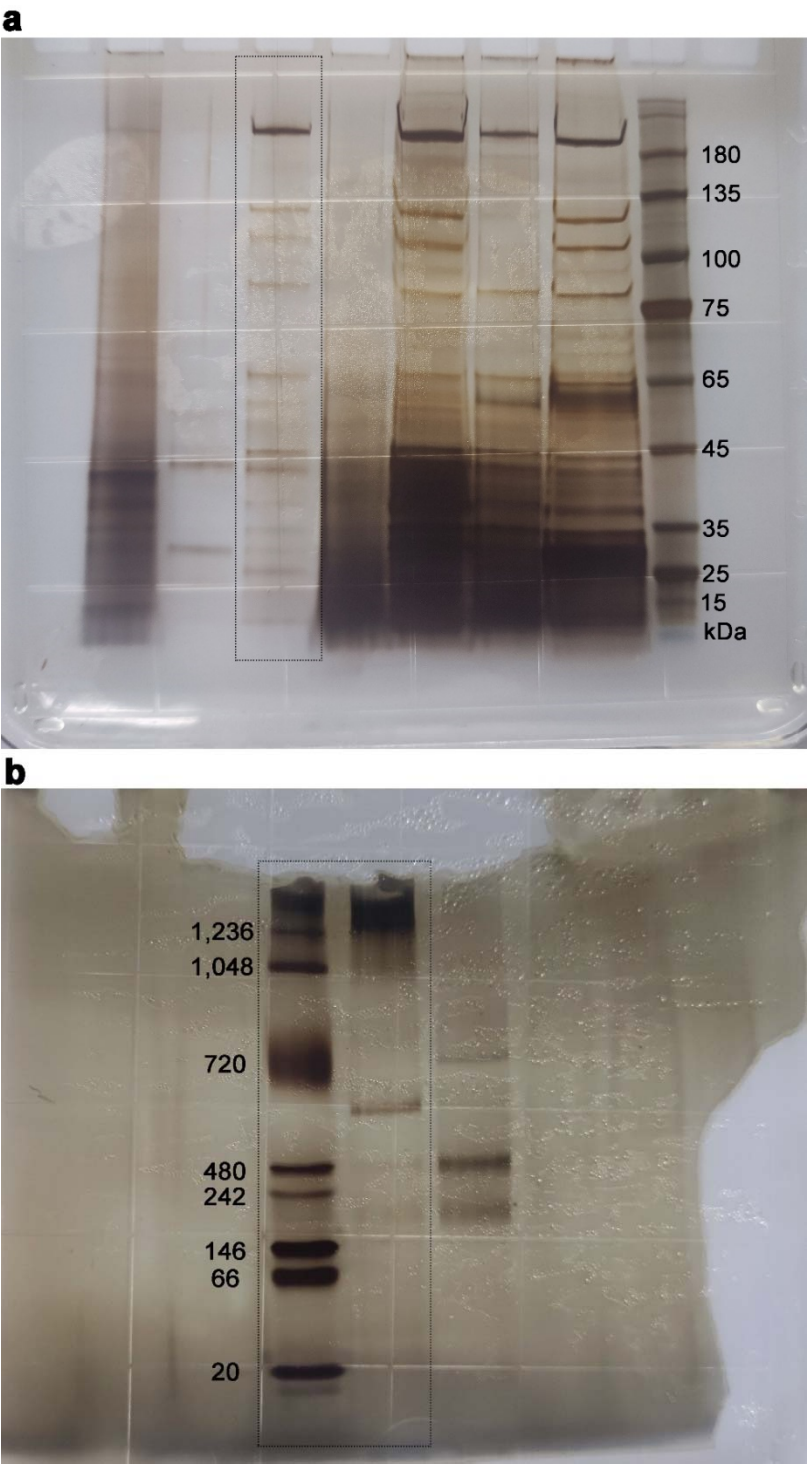

Supplementary information

**Architecture of chloroplast TOC–TIC
translocon supercomplex**

In the format provided by the
authors and unedited

Supplemental Data 1 and Table 1



Supplemental Data 1 Uncropped raw images of the SDS-PAGE and blue native PAGE gels. a, The source image for the SDS-PAGE gel presented in Extended Data Fig. 1c. **b,** The source image for the blue native PAGE gel shown in Extended Data Fig. 1d. The boxes indicate the area cropped and used for display.

Supplementary Table 1 A summary of each individual protein subunit identified in the TOC-TIC supercomplex from *C. reinhardtii*.

Gene name	Entry	Protein name	Chain ID	Length (aa)	SDS PAGE PMF score	BN PAGE unique peptides
CHLRE_06g300550v5	A0A2K3DQY7	Tic100	E	955	138	24
ycf78	P36495	Tic214	A	1995	175	77
CHLRE_17g734300v5	A0A2K3CR90	Toc90	9	967	108	19
CHLRE_03g175200v5	A8IE32	Toc75	7	798	212	22
CHLRE_12g532100v5	A0A2K3D4W3	Ctap3	3	462	139	14
CHLRE_06g252200v5	A8HYJ1; A0A2K3DM34	Toc34	G	397	74	2
CHLRE_17g722750v5	A8J6H7	Ctap4	4	363	120	8
CHLRE_03g164700v5	A0A2K3DWN5; A8IFJ3	Ctap5	5	383	49	11
CHLRE_17g727100v5	A8J6R5	Tic56	F	244	95	8
CHLRE_12g527550v5	A8J5D4	Simp2	U	124	-	4
CHLRE_09g402100v5	A8J1J3	Simp1	C	127	-	3
CHLRE_02g080250v5	A0A2K3E0K3	Simp3	D	187	-	2
CHLRE_08g379650v5	A8IZ79	Tic20	B	259	27	2

The proteins in the purified TOC-TIC supercomplex sample were subject to mass spectrometry (MS) analysis. The MALDI-TOF/TOF was used for the tryptic digestion products of individual protein bands excised from the SDS-PAGE gel, and the nano LC-Q EXACTIVE was used for the tryptic digestion products of the supercomplex band from BN-PAGE gel. The proteins were identified by considering the peptide mass fingerprinting (PMF) score, unique peptides found in the BN-PAGE band and fitting of the models in the cryo-EM densities. Note that only the proteins identified both in the cryo-EM map and mass spectrometry are included in the table. For a full list of other proteins identified in the supercomplex sample by mass spectrometry, please refer to Supplementary Table 2.

Supplementary References:

1. Kouranov, A., Chen, X., Fuks, B. & Schnell, D. J. Tic20 and Tic22 Are New Components of the Protein Import Apparatus at the Chloroplast Inner Envelope Membrane. *J. Cell. Biol.* **143**: 991-1002 (1998).
2. Kikuchi, S. *et al.* Uncovering the protein translocon at the chloroplast inner envelope membrane. *Science* **339**, 571-574 (2013).
3. Ramundo, S. *et al.* Coexpressed subunits of dual genetic origin define a conserved supercomplex mediating essential protein import into chloroplasts. *Proc. Natl. Acad. Sci. USA* **117**, 32739-32749 (2020).
4. Kessler, F. Blobel, G., Patel, H. A. & Schnell, D. J. Identification of Two GTP-Binding Proteins in the Chloroplast Protein Import Machinery. *Science*, **266**, 1035-1039 (1994).
5. Schnell, D. J., Kessler, F. & Blobel G. Isolation of Components of the Chloroplast Protein Import Machinery. *Science* **266**, 1007-1012 (1994).
6. Hiltbrunner, A. *et al.* AtToc90, a new GTP-binding component of the Arabidopsis chloroplast protein import machinery. *Plant Mol. Biol.* **54**, 427-440 (2004).
7. Bauer, J. *et al.* The major protein import receptor of plastids is essential for chloroplast biogenesis. *Nature* **403**, 203-207 (2000).
8. Kessler, F. and Blobel, G. Interaction of the protein import and folding machineries of the chloroplast. *Proc. Natl. Acad. Sci. USA* **93**, 7684-7689 (1996).
9. Lübeck, J., Soll, J., Akita, M., Nielsen, E. & Keegstra, K. Topology of IEP110, a component of the chloroplastic protein import machinery present in the inner envelope membrane. *EMBO J.* **15**, 4230-4238 (1996).
10. Chen, Y.-L. *et al.* TIC236 links the outer and inner membrane translocons of the chloroplast. *Nature* **564**, 125-129 (2018).
11. Teng, Y-S. *et al.* Tic21 Is an Essential Translocon Component for Protein Translocation across the Chloroplast Inner Envelope Membrane. *Plant Cell* **18**, 2247-2257 (2006).

12. Wu, C., Seibert, F. S. & Ko, K. Identification of chloroplast envelope proteins in close physical proximity to a partially translocated chimeric precursor protein. *J. Biol. Chem.* **269**, 32264-32271 (1994).
13. Stahl, T., Glockmann, C., Soll, J. & Heins, L. Tic40, a new 'old' subunit of the chloroplast protein import translocon. *J. Biol. Chem.* **274**, 37467-37472 (1999).
14. Küchler, M., Decker, S., Hörmann, F., Soll, J. & Heins, L. Protein import into chloroplasts involves redox-regulated proteins. *EMBO J.* **21**, 6136-6145 (2002).
15. Caliebe, A. et al. The chloroplastic protein import machinery contains a Rieske-type iron–sulfur cluster and a mononuclear iron-binding protein. *EMBO J.* **16**, 7342-7350 (1997).
16. Hörmann, F. et al. Tic32, an essential component in chloroplast biogenesis. *J. Biol. Chem.* **279**, 34756-34762 (2004).
17. Sohrt, K. and Soll, J. Toc64, a new component of the protein translocon of chloroplasts. *J. Cell. Biol.* **148**, 1213-1222 (2000).
18. Jin, Z. et al. Structure of a TOC-TIC supercomplex spanning two chloroplast envelope membranes. *Cell* **185**, 4788-4800.e4713 (2022).