**Supplementary Information**

# A Structural Study of CESA1 catalytic domain of *Arabidopsis thaliana* Cellulose Synthesis Complex: Evidence for CESA trimers

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**Figure S1**. **Multiple sequence alignment of CESA catalytic domains from *Arabidopsis thaliana* (ATCESA1CatD), *Oryza sativa* (OSCESA8catD) and *Gossypium hirsutum* (GHCESA1CatD)**. The P-CR and CSR regions are highlighted in green and yellow, respectively.

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ATCESA1         RYDRDGEPSQLVPVDVFVSTVDPLKEPPLVTANTVLSILSVDYPVDKVACYVSDDGSAML 400
OSCESA8         RYDREGEPSQLAAVDIFVSTVDPMKEPPLVTANTVLSILAVDYPVDKVSCYVSDDGAAML
GHCESA1         RYEREGEPDELAAVDFFVSTVDPLKEPPLITANTVLSILALDYPVDKVSCYISDDGAAML
                **:*:***.:*..**.*******:*****:*********::*******:**:****:***

ATCESA1         TFESLSETAEFAKKWVPFCKKFNIEPRAPEFYFAQKIDYLKDKIQPSFVKERRAMKREYE 460
OSCESA8         TFDALAETSEFARKWVPFVKKYNIEPRAPEWYFSQKIDYLKDKVHPSFVKDRRAMKREYE
GHCESA1         TFESLVETADFARKWVPFCKKFSIEPRAPEFYFSQKIDYLKDKVQPSFVKERRAMKRDYE
                **::* **::**:***** **:.*******:**:*********::*****:******:**

ATCESA1         EFKVRINALVAKAQKIPEEGWTMQDGTPWPGNNTRDHPGMIQVFLGHSGGLDTDGNELPR 520
OSCESA8         EFKVRINGLVAKAQKVPEEGWIMQDGTPWPGNNTRDHPGMIQVFLGHSGGLDTEGNELPR
GHCESA1         EYKIRINALVAKAQKTPDEGWTMQDGTSWPGNNPRD PGMIQVFLGYSGARDIEGNELPR
                *:*:***.******* *:*** *****.*****.** *********:**. * :******

ATCESA1         LIYVSREKRPGFQHHKKAGAMNALIRVSAVLTNGAYLLNVDCDHYFNNSKAIKEAMCFMM 580
OSCESA8         LVYVSREKRPGFQHHKKAGAMNALVRVSAVLTNGQYMLNLDCDHYINNSKALREAMCFLM
GHCESA1         LVYVSREKRPGYQ--KKAGAENALVRVSAVLTNAPFILNLDCD-YVNNSKAVREAMCFLM
                *:*********:*  ***** ***:********. ::**:*** *.*****::*****:*

ATCESA1         DPAIGKKCCYVQFPQRFDGIDLHDRYANRNIVFFDINMKGLDGIQGPVYVGTGCCFNRQA 640
OSCESA8         DPNLGRSVCYVQFPQRFDGIDRNDRYANRNTVFFDINLRGLDGIQGPVYVGTGCVFNRTA
GHCESA1         DPQVGRDVCYVQFPQRFDGIDRSDRYANRNTVFFDVNMKGLDGIQGPVYVGTGCVFNRQA
                ** :*:. *************  ******* ****:*::*************** *** *

ATCESA1         LYGYDPVLTEEDLEPNIIVKSCCGSRKKGKSSKKYNYEKRRGINRSDSNAPLFNMEDIDE 700
OSCESA8         LYGYEPPIKQKKKGS--FLSSLCGGRKKASKSKKKSSDKKKSNKHVDSAVPVFNLEDIEE
GHCESA1         LYGYGPPSMPSFPKS---SSSSCSCCCPGKKEPKDPSELYRDAKREELDAAIFNLREIDN
                **** *    .   .    .* *.    .... *   :  :. :: :  ..:**:.:*::

ATCESA1         GFEG--YDDERSILMSQRSVEKRFGQSPVFIAATFMEQGGIPPTTNPATLLKEAIHVISC 758
OSCESA8         GVEGAGFDDEKSLLMSQMSLEKRFGQSAAFVASTLMEYGGVPQSATPESLLKEAIHVISC
GHCESA1         YDEY-----ERSMLISQTSFEKTFGLSSVFIESTLMENGGVAESANPSTLIKEAI VISC
                  *      *:*:*:** *.** ** *..*: :*:** **:. ::.* :*:**** ****

ATCESA1         GYEDKTEWGKEIGWIYGSVTEDILTGFKMHARGWISIYCNPPRPAFKGSAPINLSDRLNQ 818
OSCESA8         GYEDKTEWGTEIGWIYGSVTEDILTGFKMHARGWRSIYCMPKRPAFKGSAPINLSDRLNQ
GHCESA1         GYEEKTAWGKEIGWIYGSVTEDILTGFKM-CRGWRSIYCMPLRPAFKGSAPINLSDRL Q
                ***:** **.****************** .*** **** * ***************** *

ATCESA1         VLRWALGSIEILLSRHCPIWYGYHGRL 845
OSCESA8         VLRWALGSVEILFSRHCPIWYGYGGRL
GHCESA1         VLRWALGSVEIFLSR------------
                ********:**::**
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**Dynamic Light Scattering (DLS)**

The hydrodynamic radii ($R_H$) of the protein samples were measured using a DynaPro NanoStar dynamic light scattering instrument (Wyatt Technology Corporation). All the measurements were carried at 21 °C. Protein samples at concentrations of 3mg/ml were filtered using 0.22-µm filters and centrifuged for 15 min at 18000 x g before the data was collected. For each sample 10 measurements were made with an acquisition time of 10 seconds each. The data were analyzed using the Dynamics V6 software provided by instrument manufacturer. All the samples were assumed to be globular and spherical for analysis.

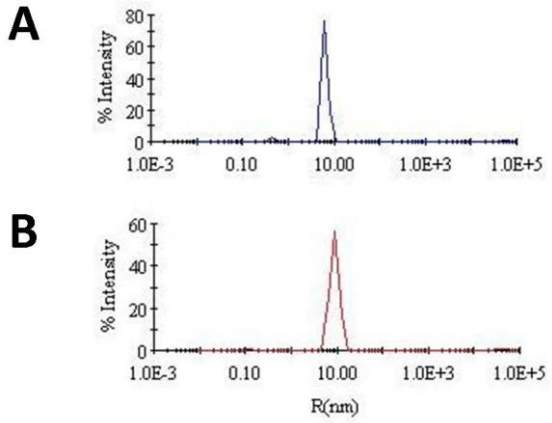



**Figure S2. Dynamic light scattering studies of ATCESA1CatD.** (A) The purified ATCESA1CatD in presence of sodium lauroyl sarcosine is shown to exist as monodisperse protein with a hydrodynamic radius of ~ 5.1 nm. (B) ATCESA1CatD upon removal of lauroyl sarcosine by dialysis is shown to be a monodisperse protein with a hydrodynamic radius of ~ 8.4 nm.

**Figure S3. Guinier plot and fit from the scattering data of ATCESA1CatD monomer.** The fit curve is the solid black line. The curve has been offset for clarity. $Q_{max}.R_g$ for the fit is 1.27.

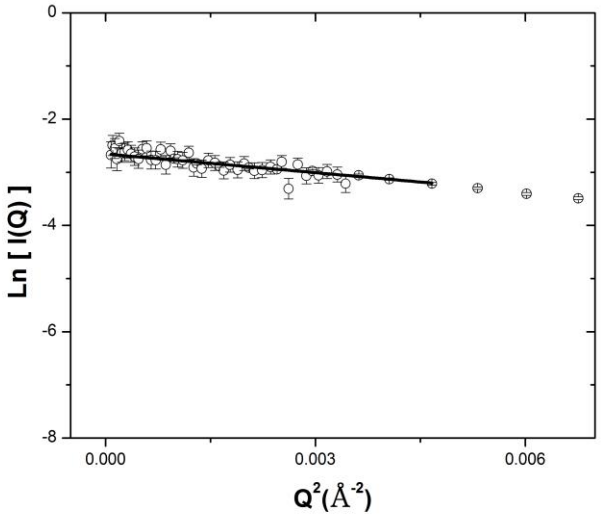


**Figure S4. Kratky plot derived from scattering data of ATCESA1CatD monomer.**

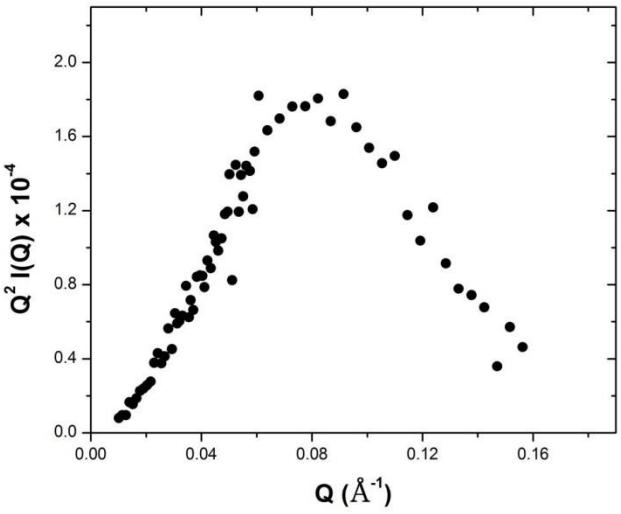


**Figure S5. Guinier plot and fit from the scattering data of ATCESA1CatD trimer.** The fit curve is the solid black line. $Q_{max}.R_g$ for the fit is 1.28.

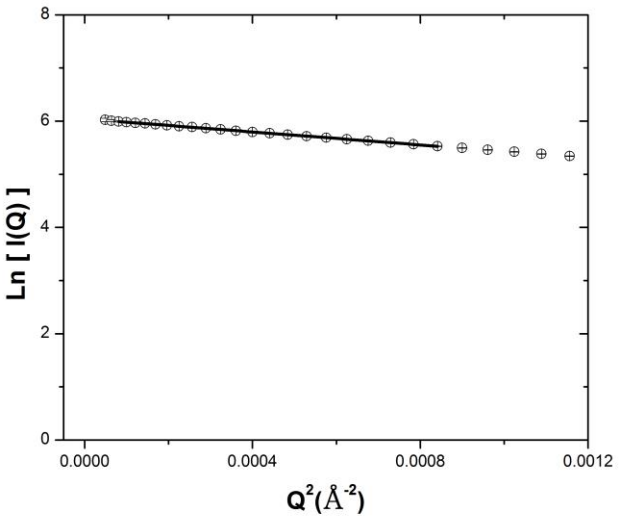
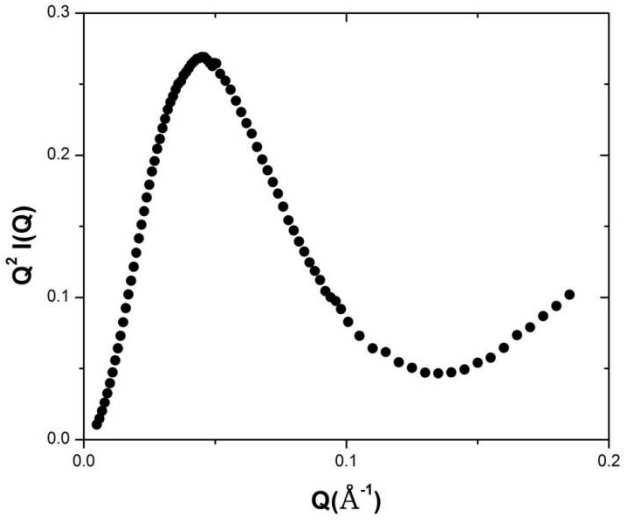


**Figure S6. Kratky plot derived from scattering data of ATCESA1CatD trimer.**



**Estimation of dimensions of individual lobes at the cytosolic side of a Rosette cellulose synthase complex**

The dimensions of the individual lobes of the cytosolic side of a rosette cellulose synthase complex (CSC) were estimated based on previously reported data from transmission electron microscopy (TEM) where the transmembrane helices (TMH) across the plasma membrane, and assuming that the arrangement of the lobes is the same on both sides of the membrane.

TEM images of the TMH of the CSC, as viewed in metal replicas prepared by freeze fracture TEM (FF-TEM), show a complex with approximately 24 nm overall diameter and 8 nm diameter individual lobes [1-3]. If each lobe of the CSC is represented by a circle with a diameter (*d*) of 8 nm and the center of each circle is placed at a vertex of a regular hexagon, then the length of each facet is equal to *d* and the maximum dimension of the rosette CSC is 3*d* (or 24 nm). The schematic arrangement of 6 circles arranged in a P6 symmetry is shown in Figure S7, overlaid with a FF-TEM image of the rosette CSC, as viewed in the extracellular surface of the plasma membrane, excerpted from Fig. 8 of reference [4].

Bowling and Brown reported that the cytosolic side of the CSC has a hexagonal shape and maximum dimension of 45-50 nm [5]. Assuming that the arrangement of the lobes of the CSC are the same on both sides of the membrane, then *d*, the diameter of an individual lobe can be estimated to be 15 – 16 nm at the cytosolic side of the membrane.

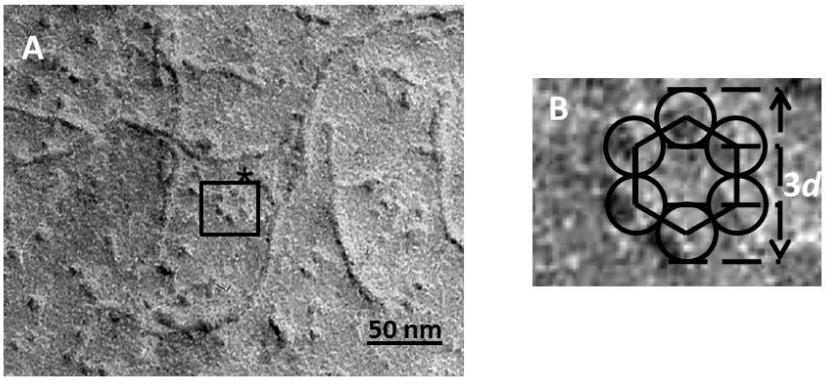



**Figure S7. Schematic of hexagonal arrangement of lobes of a rosette CSC overlaid with TEM image of the TMH of rosette CSC.** The TEM image was adapted from Haigler et al, 2014 [4]. The box in panel A represents the area presented in panel B.

## References


1. Rudolph, U., H. Gross, and E. Schnepf, *Investigations of the Turnover of the Putative Cellulose-Synthesizing Particle Rosettes within the Plasma-Membrane of Funaria-Hygrometrica Protonema Cells .2. Rosette Structure and the Effects of Cycloheximide, Actinomycin-D, 2.6-Dichlorobenzonitrile, Biofluor, Heat-Shock, and Plasmolysis.* Protoplasma, 1989. **148**(2-3): p. 57-69.
2. Rudolph, U. and E. Schnepf, *Investigations of the Turnover of the Putative Cellulose-Synthesizing Particle Rosettes within the Plasma-Membrane of Funaria-Hygrometrica Protonema Cells .1. Effects of Monensin and Cytochalasin-B.* Protoplasma, 1988. **143**(1): p. 63-73.
3. Mueller, S.C. and R.M. Brown, *Evidence for an intramembrane component associated with a cellulose microfibril-synthesizing complex in higher plants.* J Cell Biol, 1980. **84**(2): p. 315-326.
4. Haigler, C.H., et al., *Molecular Modeling and Imaging of Initial Stages of Cellulose Fibril Assembly: Evidence for a Disordered Intermediate Stage.* Plos One, 2014. **9**(4).
5. Bowling, A.J. and R.M. Brown, *The cytoplasmic domain of the cellulose-synthesizing complex in vascular plants.* Protoplasma, 2008. **233**(1-2): p. 115-127.