EVIDENCE OF A TRANSITION FROM DIFFUSIVE TO SUPER-DIFFUSIVE MOTION OF CELLULOSE SYNTHASE COMPLEXES IN LIVING PLANTS

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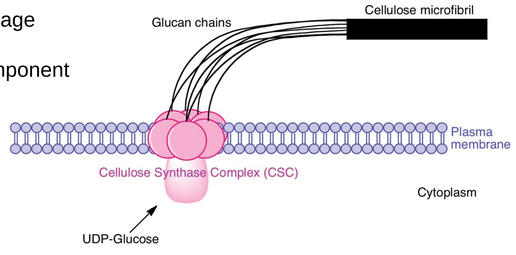
Introduction

The polysaccharide cellulose is the main component of plant cell. The large transmembrane protein complex responsible for synthesizing cellulose contains several cellulose synthase A (CESA) subunits that polymerize UDP glucose into the constituent glucan chains of cellulose. Here, we used variable angle epi-fluorescence microscopy in combination with single-particle tracking to characterize the motion of GFP labeled CESA complexes in the mesocotyl of *Brachypodium distachyon* seedlings that are a 3 to 4 days old. We show that CESA complexes move through the plasma membrane at approximately 165 nm/minute. Their motion is known to be partially guided by cortical microtubules, but no molecular motors are involved. Rather, the motion is thought to be driven by the polymerization and crystallization of the cellulose. A mean-squared displacement analysis shows that CESA complexes move diffusively on short time scales and undergo a transition to super-diffusive motion. We also report on the effect of actin and microtubule inhibitors.

Cellulose synthase(CESA) complex

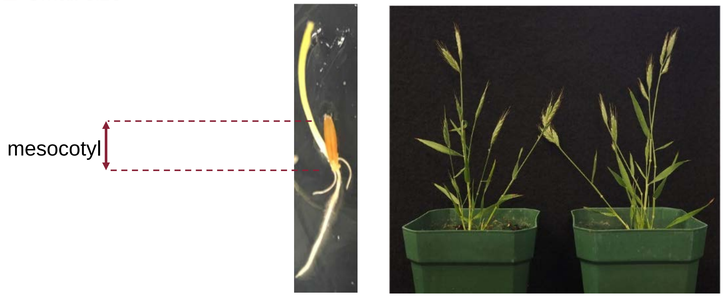
- catalyze the β 1-4 linkage
- have 6 subunits
- CESA is the main component



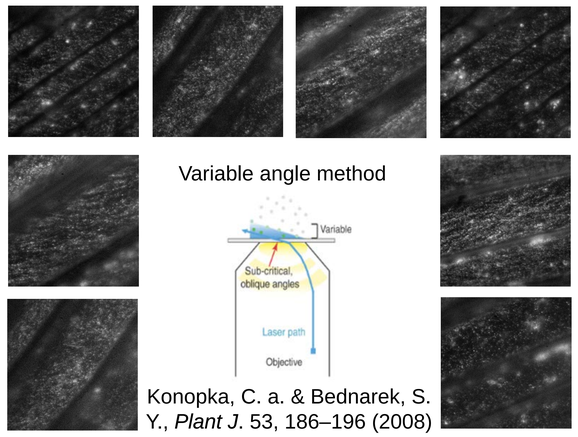
Wightman, R., & Turner, S. *Biochem. Soc. Trans.*, 38, 755–760 (2010);

Model organism: Brachypodium distachyon (B. distachyon)

- Grasse
- significant and renewable source of energy
- short genome sequence
- rapid lifecycle
- Small size



Observing single complexes in living plants cells using Variable angle Epi-fluorescence Microscopy (VAEM)

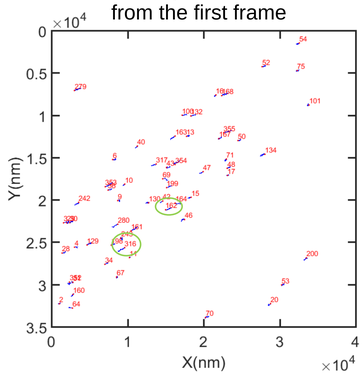


Variable angle method

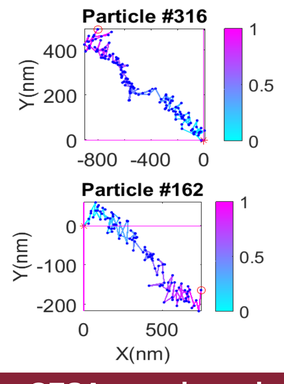
Konopka, C. a. & Bednarek, S. Y., *Plant J.* 53, 186–196 (2008)

Particle tracking

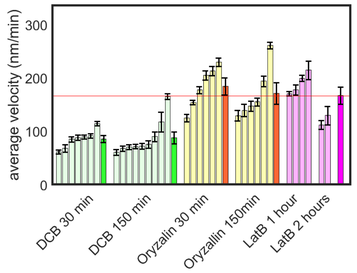
Particles' trajectories that start from the first frame



Bidirectional motion



The effect of various inhibitors on CESA complex velocity



- 2,6-dichlorobenzonitrile (**DCB**): Cellulose synthase inhibitor
- Oryzalin: Microtubule formation inhibitor
- latrunculin B (**Lat B**): Actin assembly inhibitor

The solid red line shows the average velocity of CESAs in the untreated mesocotyl.

Mean Squared Deviation of CESA trajectories

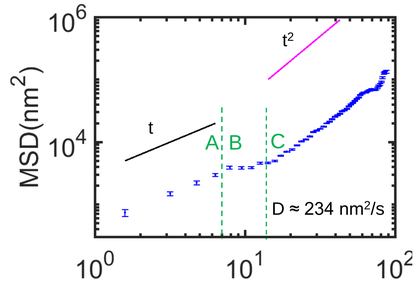
$$MSD = \langle |r_i(t+\tau) - r_i(\tau)|^2 \rangle - 2\sigma^2$$

Measurement of MSD is corrected for localization error $2\sigma^2$

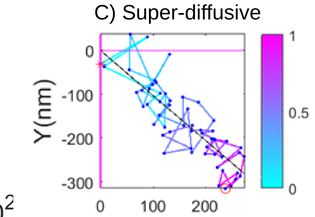
$MSD = 2Dt^{\alpha}$

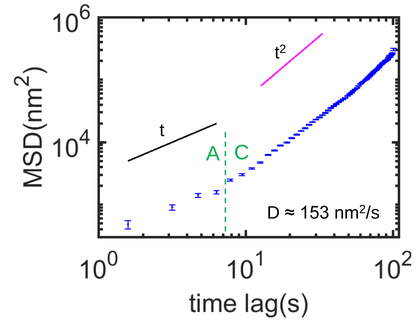
- α=1 normal diffusion
- α<1 sub-diffusion
- α>1 super-diffusion

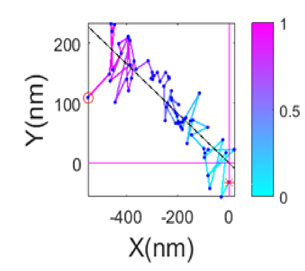
MSD of two representative CESA complexes in one dimension, along the principal axis of motion



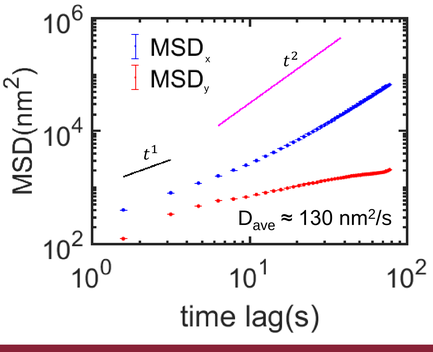
A) Diffusive
B) Trap effect
C) Super-diffusive



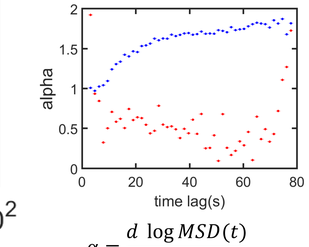




Average MSD for 2186 CESA particles



Blue points are one dimensional MSD along the principal axis of the trajectory



$$\alpha = \frac{d \log MSD(t)}{d \log t}$$

Acknowledgments

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