SUPPLEMENTARY MATERIALS

For

Dynamic mechanical responses of *Arabidopsis* thylakoid membranes during PSII-specific illumination

by C. H. Clausen et al.

Figure S1

Figure S2

Figure S3

Figure S4

Figure S5

Figure S6

Derivation of Equation 1

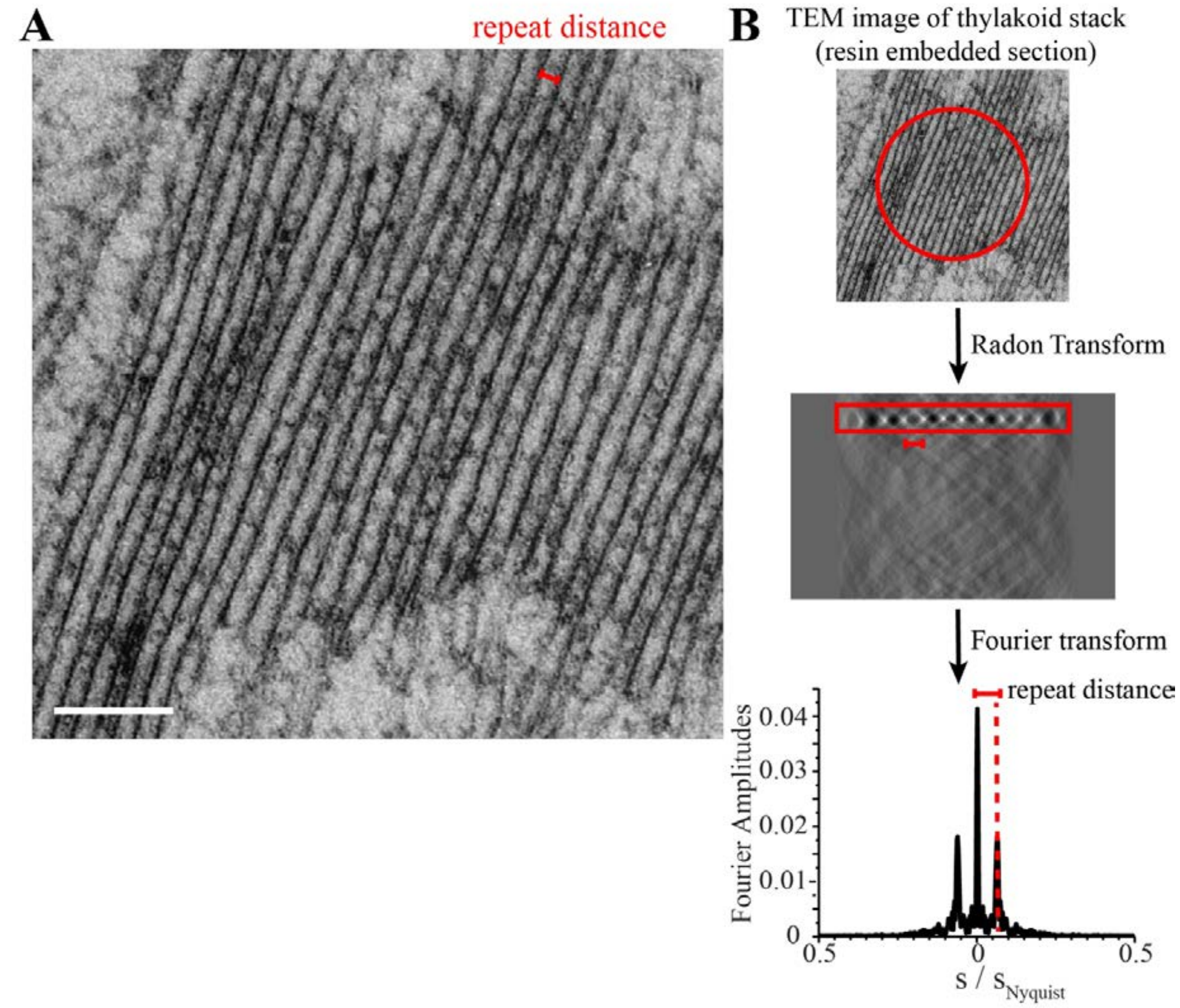

Figure S1: Stacking repeat distance measurements from EM images (resin embedded sections) (A) Detail of a TEM image of high-pressure frozen freeze-substituted section through a thylakoid stack showing the characteristic repeat motif. The repeat marked in red includes two membrane bilayers, the stromal and the lumenal spaces. As illustrated here, the stromal space or "partition gap" cannot be distinguished from the membrane bilayers (dark stripe). (B) Repeat distance measurement: the characteristic stacking repeat was measured for each thylakoid (top) by finding the peaks in the Fourier transform of its Radon transform. Scale bar is 100nm.

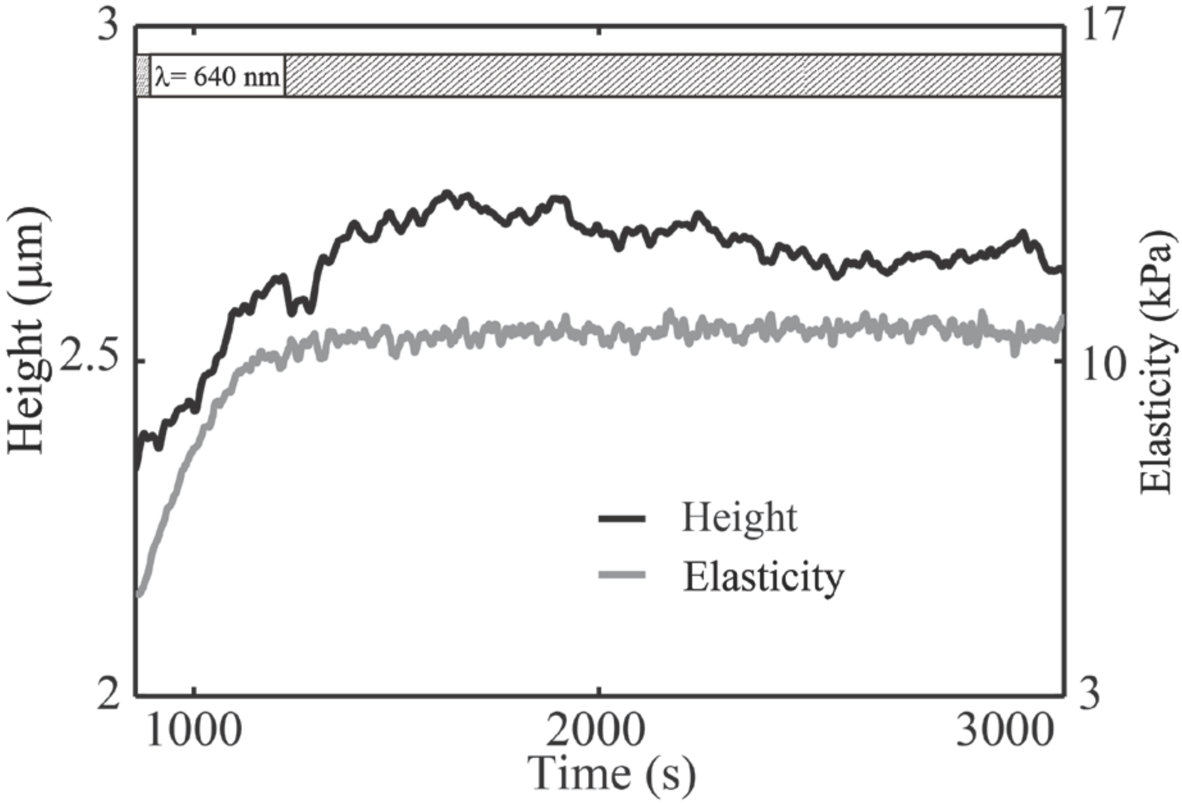

Figure S2: Plot of the normalized stiffness and height of a wild-type thylakoid membrane illuminated for 6 min followed by 30 min in the dark. Neither the stiffness nor the height show a significant change after λ=640 nm illumination is turned off within this time span.

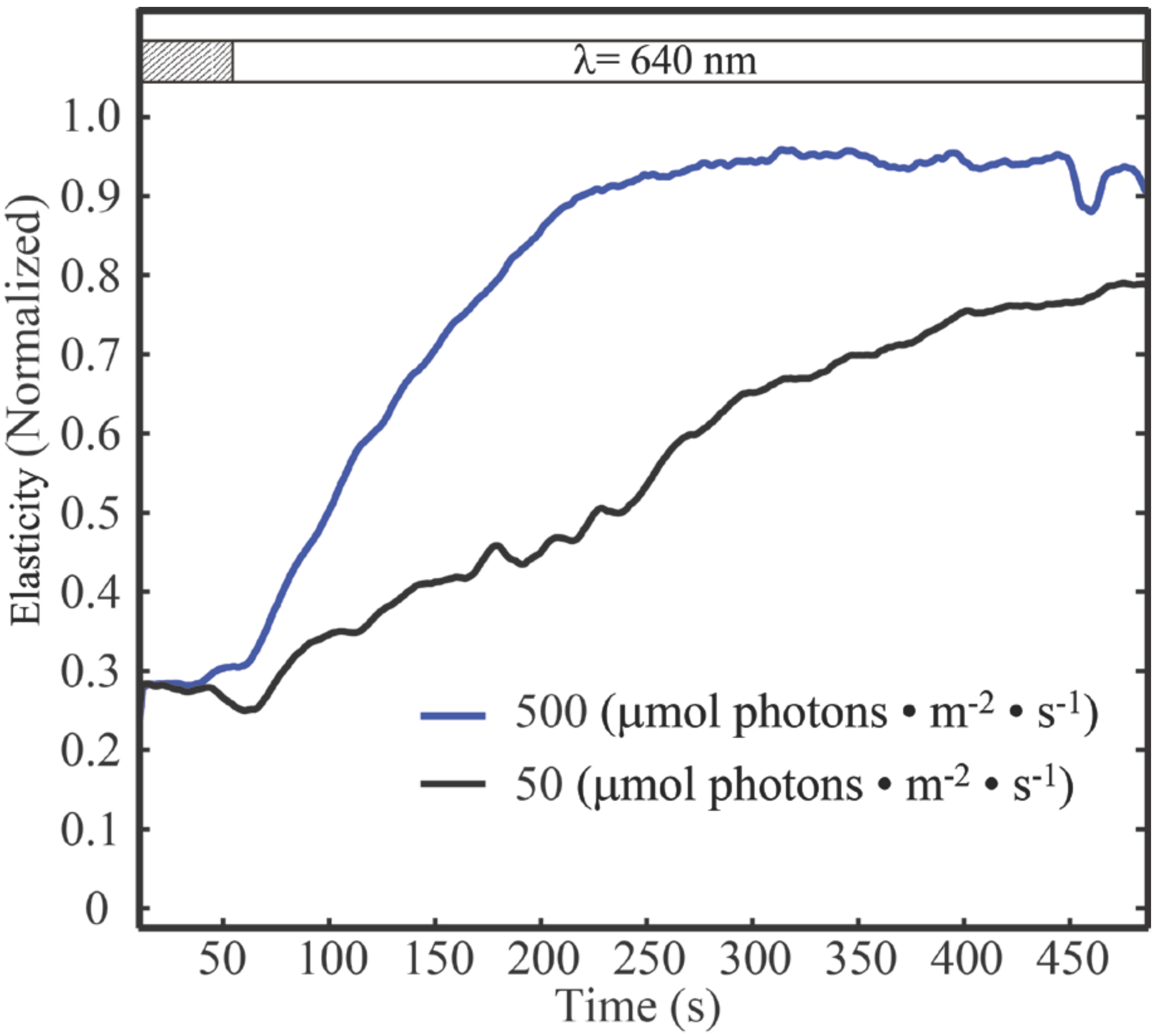

Figure S3: Plot of the normalized stiffness from wild-type thylakoid membranes illuminated with 640 nm light at different intensities. The plot shows that the stiffness response time (t_r) is greater at higher light intensity.

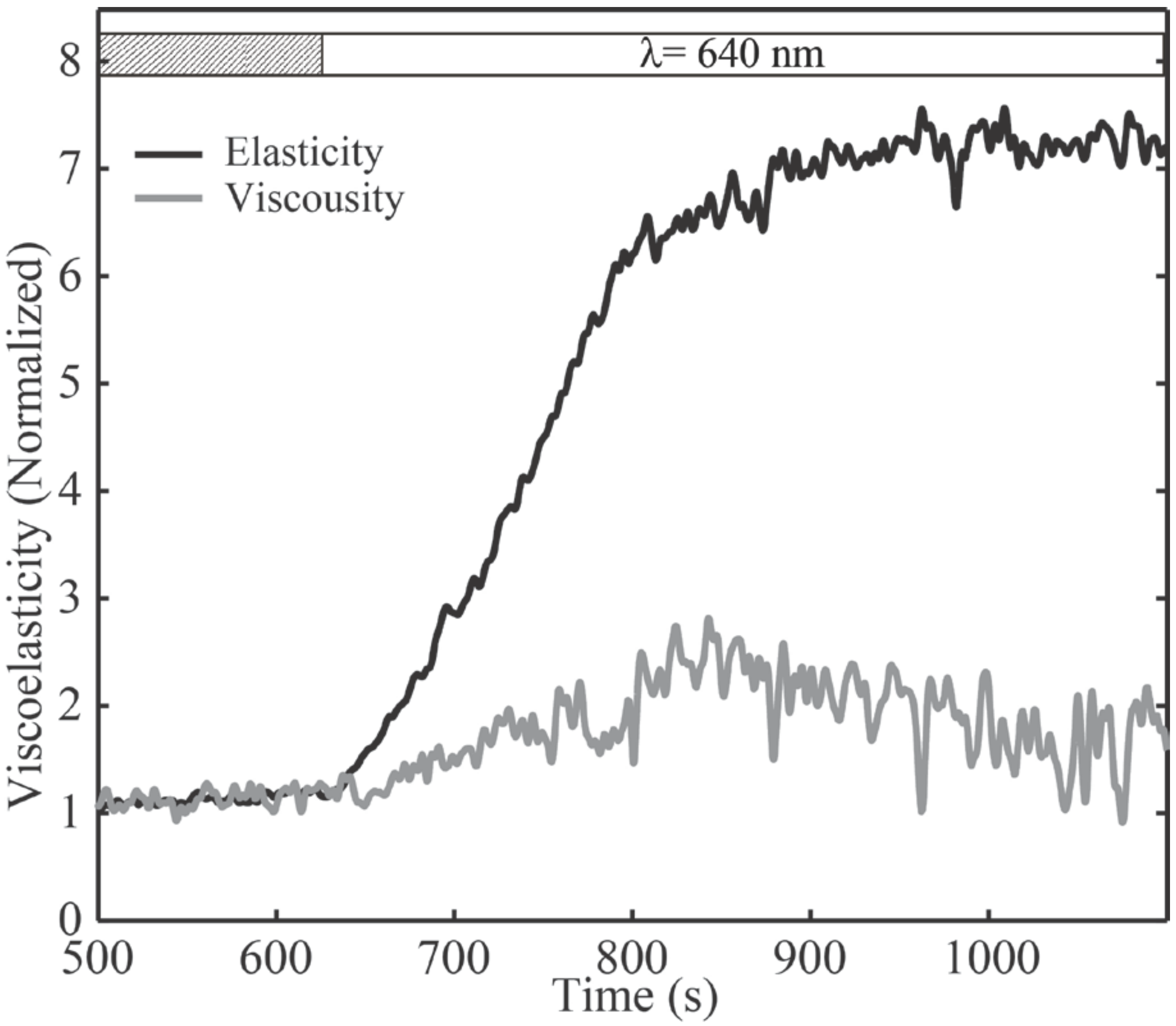

Figure S4: The viscoelasticity of a single measurement calculated according to Eq. 1. The elastic and viscous moduli are normalized to their initial values. Changes in G" in response to λ=640 nm illumination are less significant than in G'.

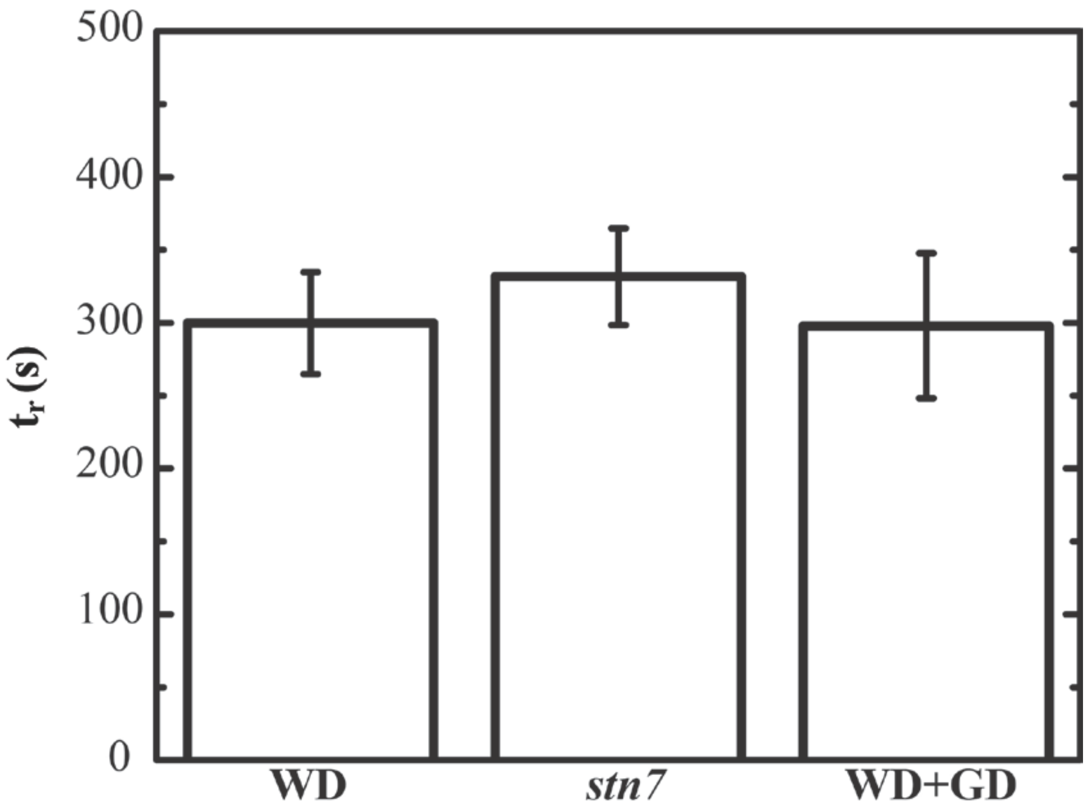

Figure S5: Histogram plot of the stiffness response time (t_r) in seconds for wild type (WT), *stn7*, and wild type treated with gramicidin D (WT + GD). The response time does not depend on the presence of STN7 or formation of the pH gradient.

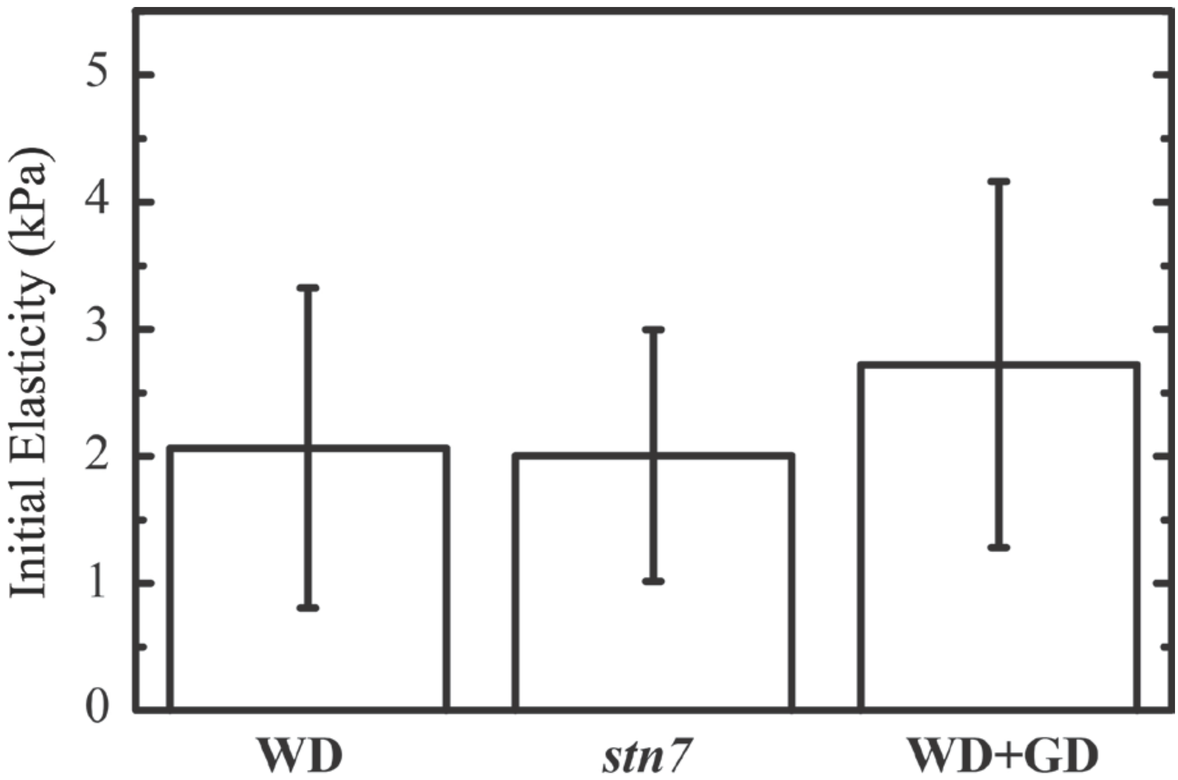

Figure S6: The initial elasticity (G') of wild type (WT), *stn*7 mutant, and gramicidin D (GD) treated thylakoid membranes before PSII illumination. There is no significant difference between all different samples.

Derivation of Equation 1:

Using Equation 5 from Mahaffy *et al.* 2004

$$K_1^* = \frac{f_{osc}^*}{2\delta^*(R\delta_0)^{\frac{1}{2}}}$$

combined with the definitions $K = \frac{E}{1-v^2}$ and $G = \frac{E}{2(1+v)}$, we obtain

$$G^* = \frac{(1-v)f_{osc}^*}{4\delta^*(R\delta_0)^{\frac{1}{2}}} ,$$

where f_{osc}^* and δ^* are complex numbers describing force and displacement, respectively, that depend on angular frequency ω and phase ϕ. For negligible drag, which we confirmed for the frequencies used in our experiments, the applied force is given by

$$f_{osc}^* = k \cdot A \cdot \exp\big(i(\omega t + \phi)\big) ,$$

where k is the spring constant of the cantilever and A is the measured cantilever amplitude. The indentation is given by

$$\delta^* = \delta \cdot \exp(i\omega t) ,$$

where δ is the applied amplitude minus the measured amplitude. Combining the equations for G^*, $f_{osc}{}^*$, and δ^* , we obtain

$$G^* = \frac{(1-v)*k*A*\exp\big(i(wt+\phi)\big)}{4*\delta*\exp(iwt)*(R\delta_0)^{\frac{1}{2}}} = \frac{(1-v)\cdot k\cdot A}{4\cdot\delta\cdot\sqrt{R\delta_0}} \cdot \exp(i \cdot \phi) ,$$

which is the form used in Equation 1 in the text.