Constitutive and regulatory responses of *Arabidopsis thaliana* to harmonically oscillating light

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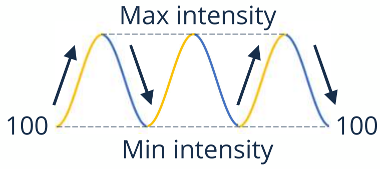
Studied mutants and conditions

Genotypes & Mutants

- **Col-0** (Complete qE system: active PsbS protein and xanthophyll cycle)
- ***npq1*** (Vio. de-epoxidase (VDE) inactive → no Zea-dependent qE)
- ***npq4*** (PsbS protein absent → no PsbS-dependent qE mechanism)

Light Oscillation Conditions

- **Period** (s): 1, 5, 10, 30, 60, 120, 240, 480
- **Amplitude** (μmol m^{-2} s^{-1}): 100–200, 100–400, 100–800

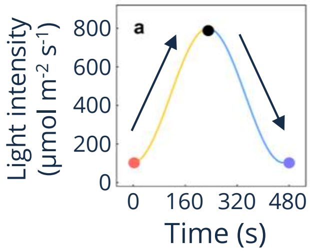


Experimental Protocol

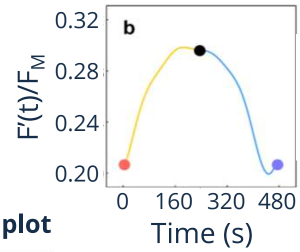
- **Instrument**: Dual-KLAS-NIR + 3010-DUAL leaf cuvette (each 0.1 s)
- **Pre-acclimation:** 10 min at mean irradiance of upcoming oscillation
- **Replicates**: 3 plants per genotype and amplitude conditions

Relationship of photosynthesis and light intensity under oscillating light conditions

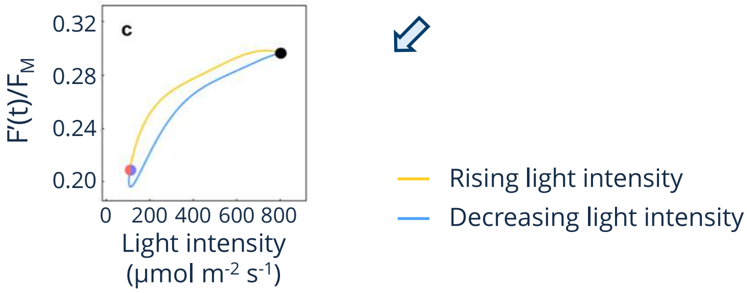
System input - Light oscillation



System output - ChlF



Input-output plot



- Oscillation period: <u>8 min</u>
- Oscillation amplitude: <u>100 – 800</u> µmol m^{-2} s^{-1}

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Multiple non-linear responses to changes in light intensity

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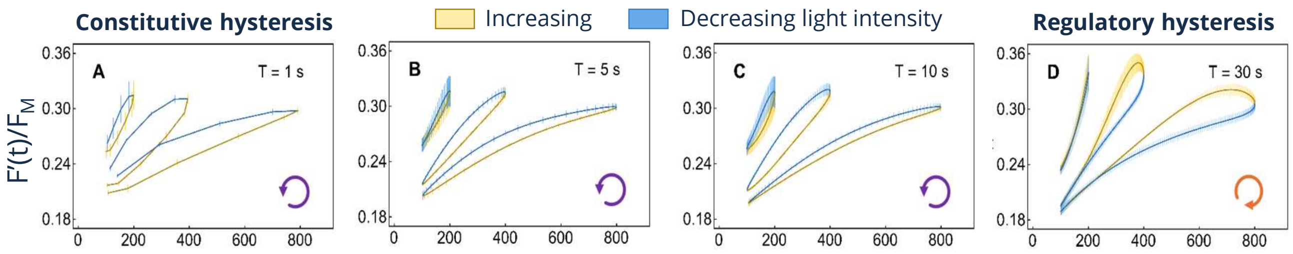
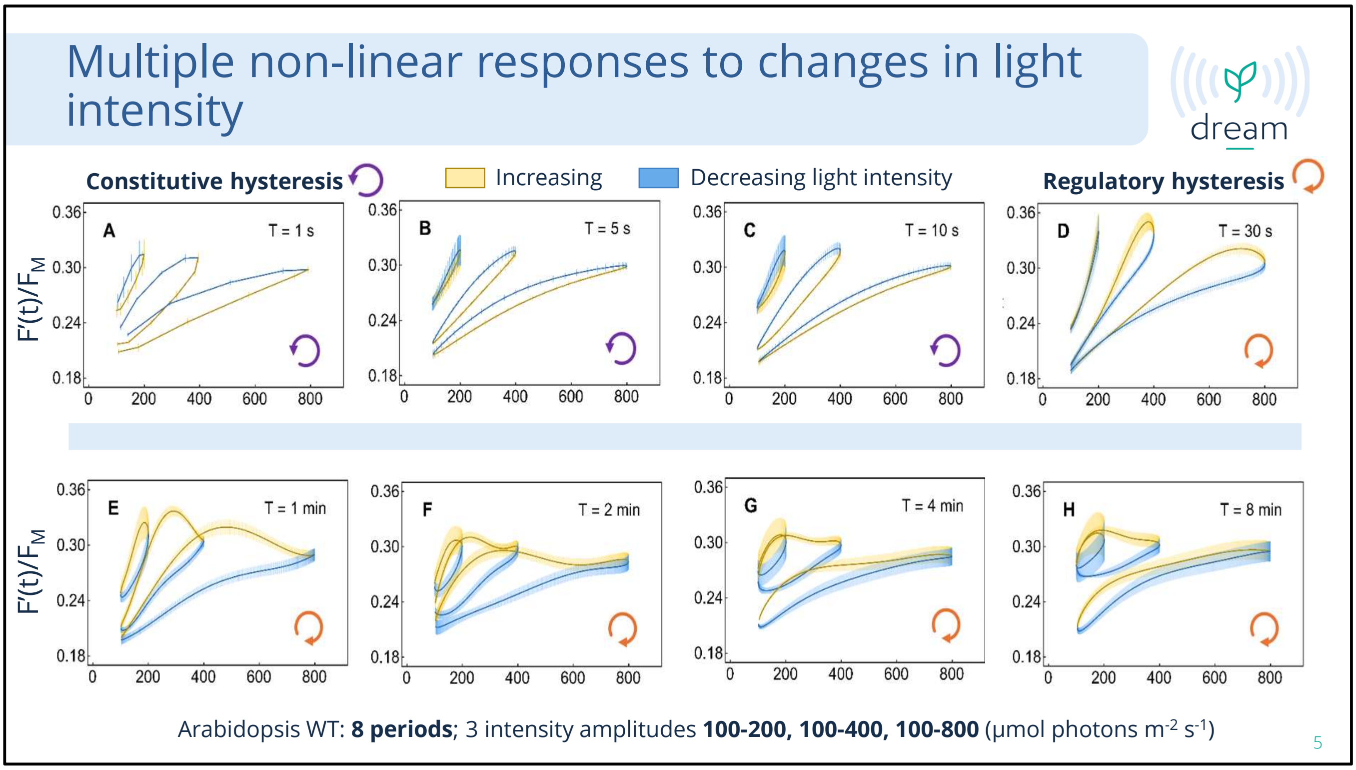


- Two distinct hysteresis types identified
  - Constitutive hysteresis: Occurs in rapid oscillations (T ≤ 10s), independent of NPQ
  - Regulatory hysteresis: Dominates in slower oscillations (T > 30s), requires PsbS-dependent qE

When it comes to 30s, very important phenomenon is that the orientation of loops shifted from counterclockwise to clockwise**. It seemingly looks like the action of response is before the stimulation, we call this phenomenon regulatory hysteresis**. It stated in 30s and became exploded in 1min, exhibiting a clear signal decline in light rising phase. **We believe that this regulatory hysteresis is induced by the onset of dynamic NPQ regulation.** This will be explained later.



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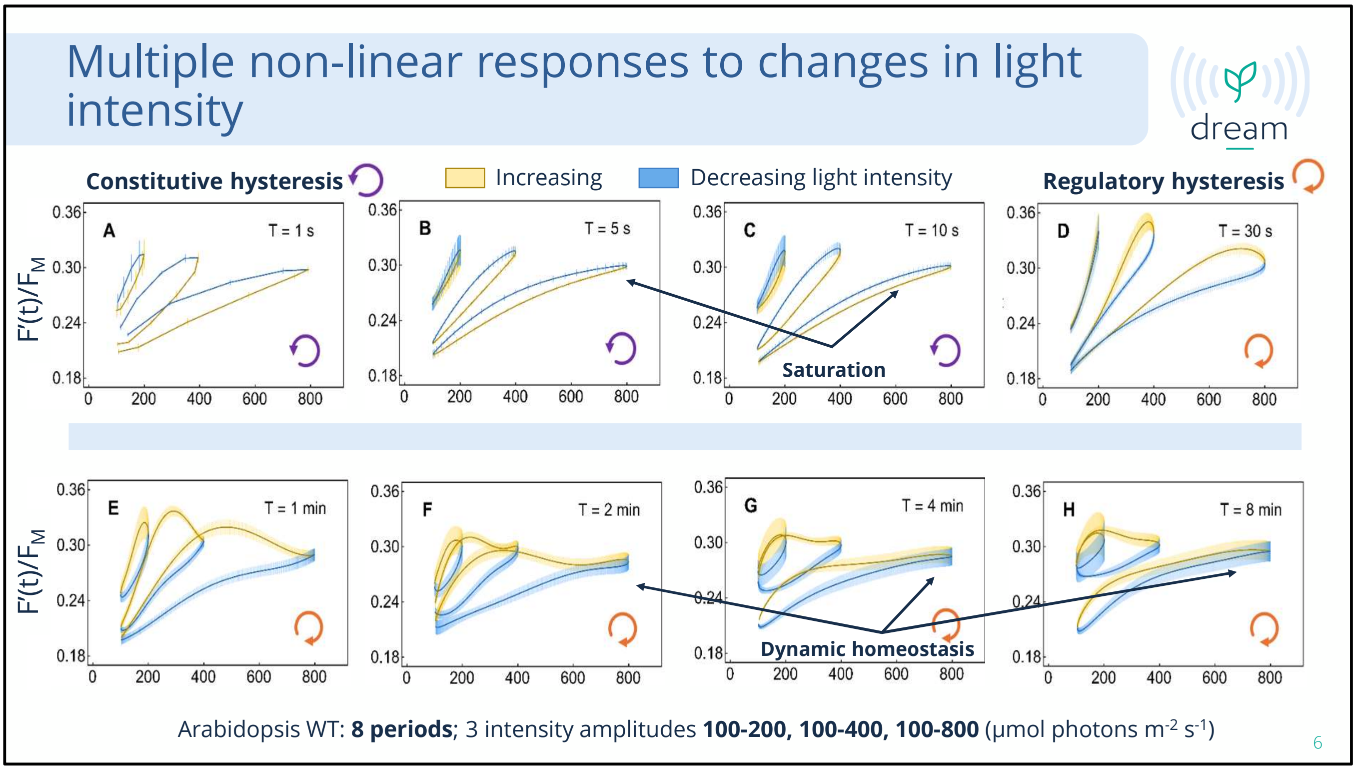

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Constitutive hysteresis & saturation are not caused by NPQ

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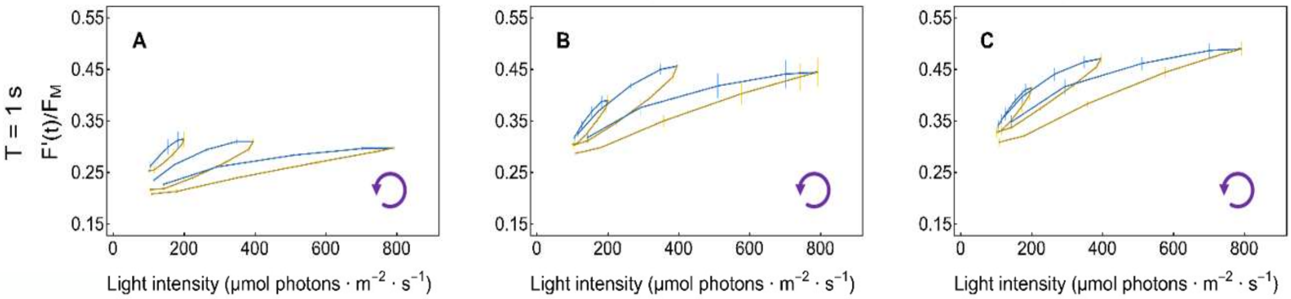
Col-0 | ***npq1* (no VDE-NPQ)** | ***npq4* (no PsbS-NPQ)**



T = 1 s

- Constitutive hysteresis & saturation also occur in mutants (primary reactants cause the hysteresis)

Regulatory response is induced by PsbS-NPQ

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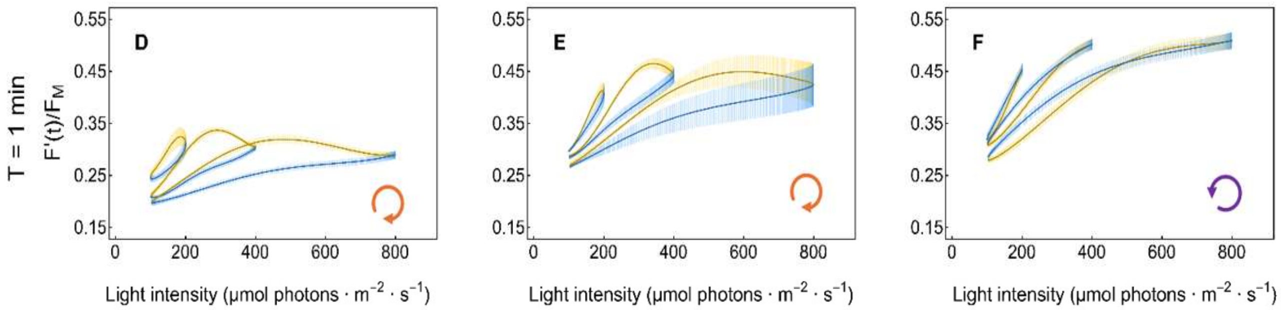
Col-0 | ***npq1* (no VDE-NPQ)** | ***npq4* (no PsbS-NPQ)**



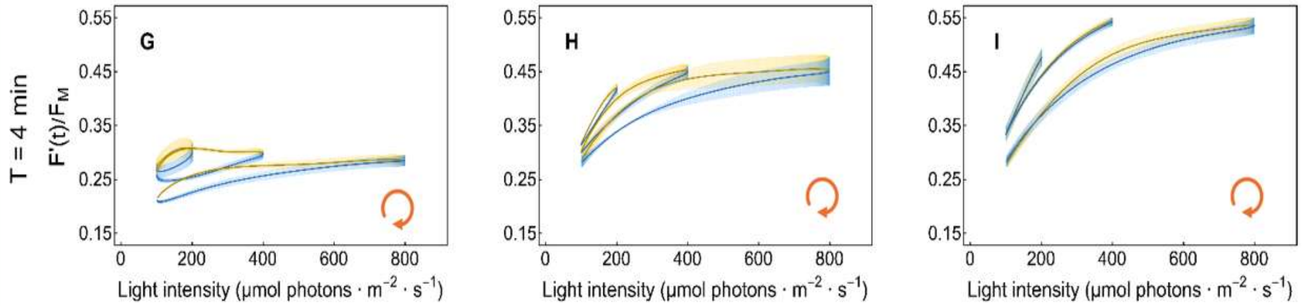
T = 1 min

- Regulatory response disappears in *npq4* but is present in *npq1* (hysteresis loop is absent)

Why yellow going up and then down

Regulatory response is induced by PsbS-NPQ

Col-0 | ***npq1* (no VDE-NPQ)** | ***npq4* (no PsbS-NPQ)**



T = 4 min

- Both NPQ processes are essential in response to longer periods of oscillating light (homestasis barely kept)

Downshift in npq4 due to probable accumulation of Zea?

Fluorescence definition *in silico*

$$\frac{F'(t)}{F_M} = \frac{F_0{}'(t) + F_V{}'(t)}{F_M} = \frac{F_0{}'(t) + \left(F_M{}'(t) - F_0{}'(t)\right) \cdot RCII_{closed}(t)}{F_M}$$

BDM2 Fluorescence equation

$$\frac{F_M{}'(t)}{F_M} = \left(1 - PsbS_{max} PsbS_{act}(t)\right)\left(1 - Zea_{max} Zea(t)\right)$$

Defined in the BDM2 model

$$\frac{F_0{}'(t)}{F_M} = \frac{\frac{F_M{}'(t)}{F_M}}{1 + \frac{\emptyset_{IImax}}{1 - \emptyset_{IImax}} \frac{F_M{}'(t)}{F_M}}$$

Oxborough and Baker (1997)

$$F'(t)/F_M = \left(1 - Zea_{max} \cdot Zea(t)\right)\left(1 - PsbS_{max} \cdot PsbS_{act}(t)\right)\left[\frac{1 - RCII_{closed}(t)}{1 + \frac{\Phi_{II,max}}{1 - \Phi_{II,max}}\left(1 - Zea_{max} \cdot Zea(t)\right)\left(1 - PsbS_{max} \cdot PsbS_{act}(t)\right)} + RCII_{closed}(t)\right]$$

Comparing simulations and exp. fluorescence (WT)

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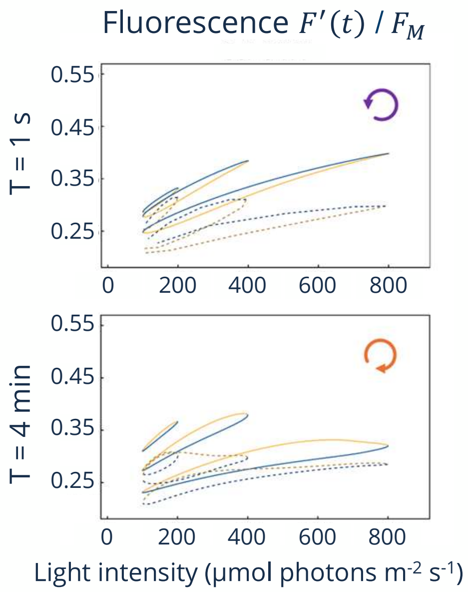

Model Experiment

- Model agrees just qualitatively (slightly overestimation)
- Fluorescence decreases with larger light amplitudes
- Hysteresis orientation predicted
- Poorer modelling results at 4 min (homestasis not predicted)

What can be the reason behind the homestasis at 4 min)

Comparing simulations and exp. fluorescence (*npq1*)

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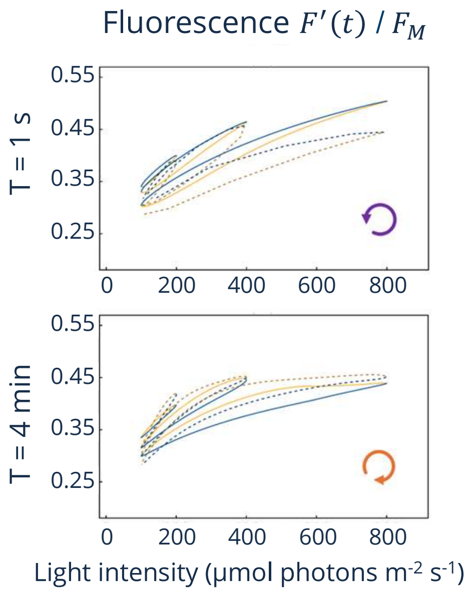
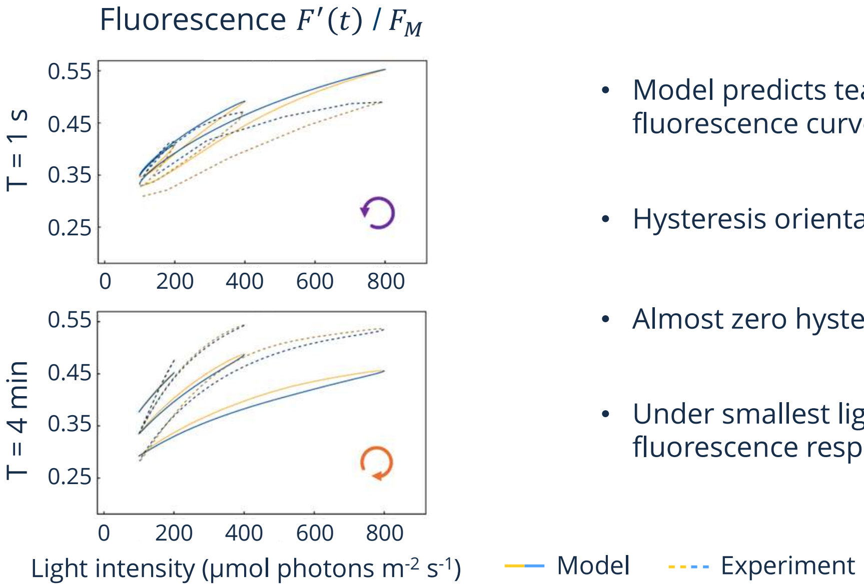


Model Experiment

- Model agrees qualitatively
- However, under very high light, largest F′(t) value predicted in silico)
- Hysteresis orientation predicted
- Good approximation at 4 min
- Under smallest light amplitude, fluorescence responds linearly

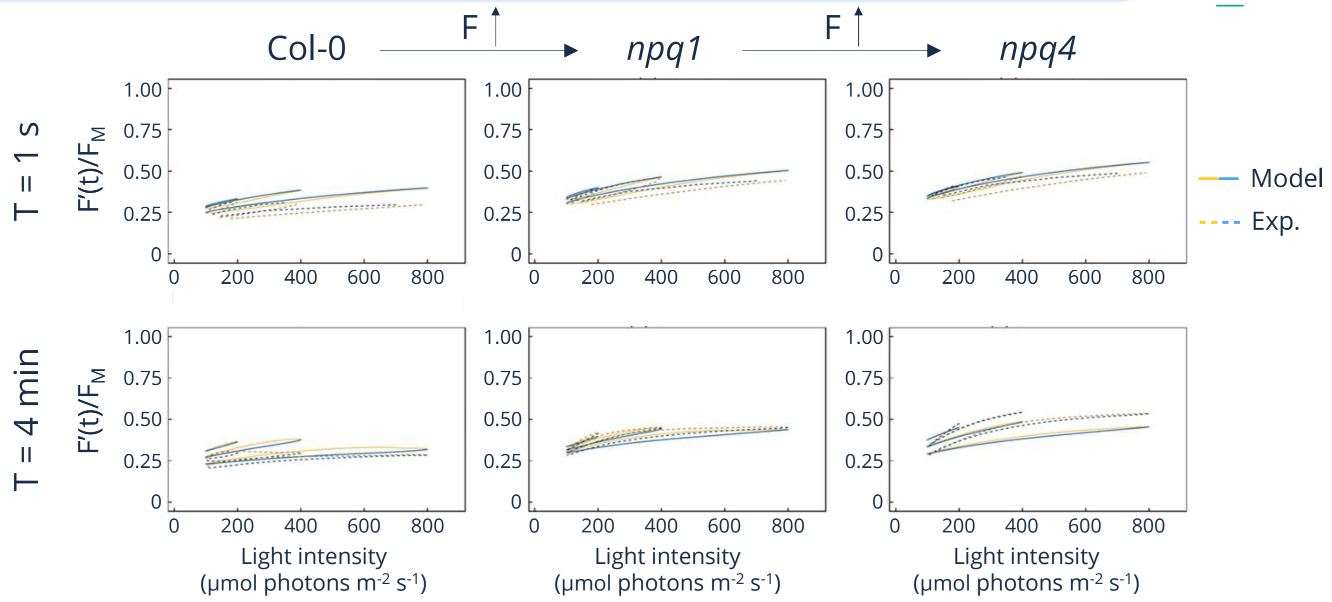
Why hysteresis at 4 min

Comparing simulations and exp. fluorescence (*npq4*)



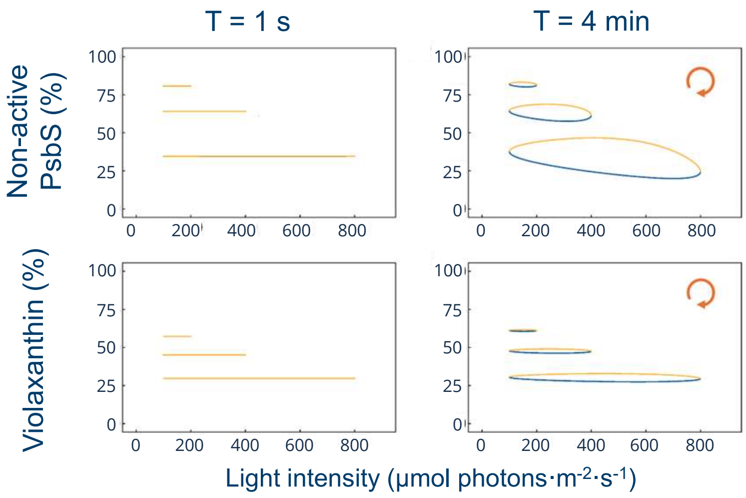
- Model predicts tear-shaped fluorescence curves
- Hysteresis orientation predicted
- Almost zero hysteresis estimated
- Under smallest light amplitude, fluorescence responds linearly

Comparing simulations and exp. fluorescence (scale zoomed out)



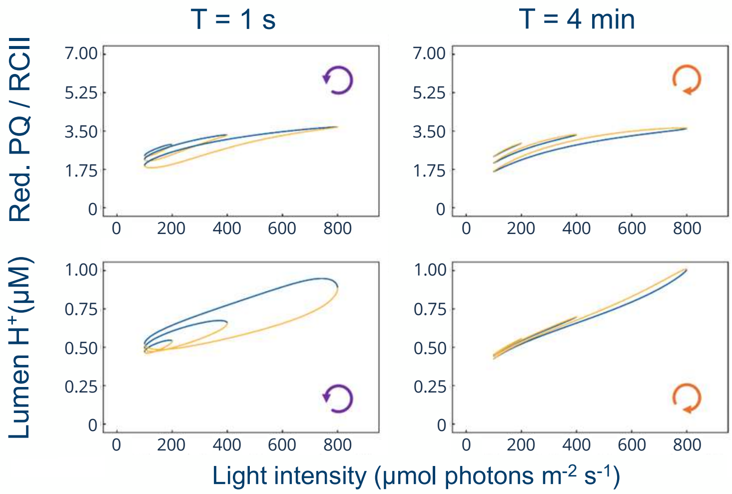
Zoom out shows an acceptable agreement int terms of mean values

Simulation of other variables (WT)



- Non-active PsbS and violaxanthin remain constant at 1 s
- Violaxanthin almost constant at 4 min
- Transient increase in non-active PsbS at 4 min (NPQ has a delay, max. ChlF before max. intensity)
- Hysteresis orientation predicted

Simulation of other variables (WT)



- PQ pool 25-50% reduced
- PQ pool less reduced under quickly than slowly rising light
- PQ pool reduction driven by NPQ at 4 min
- Lumen H^+ shows a clear hysteresis loop at 1 s
- Nearly linear relationship of H^+ vs light intensity at 4 min

Why pH is linear at 4 min

Conclusions

- Rather acceptable agreement with data though model simplicity
- Two distinct hysteresis types identified
  - Constitutive hysteresis: Occurs in rapid oscillations ($T \leq 10s$), independent of NPQ
  - Regulatory hysteresis: Dominates in slower oscillations ($T > 30s$), requires PsbS-dependent qE
- Both NPQ mechanisms are important to keep homestasis under high light
  - *npq4* regulatory response absent at 1 and 4 min (lacks PsbS-dependent qE)
- Future work
  - Extend frequency range to identify additional regulatory mechanisms
  - Incorporate other regulatory mechanisms, improve NPQ characterisation

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Use model-experiment discrepancies to identify knowledge gaps in photosynthetic regulation dream

Thanks for your attention!