Introduction to Genetically Encoded Biosensors

Why Use Genetically Encoded Biosensors?

Biosensors (‘biological sensors’) are biological tools that monitor a process or detect a given molecule. The sensor component is usually a protein that undergoes a conformational change in response to the molecule it detects.

- High selectivity
- Definite quantification
- High resolution

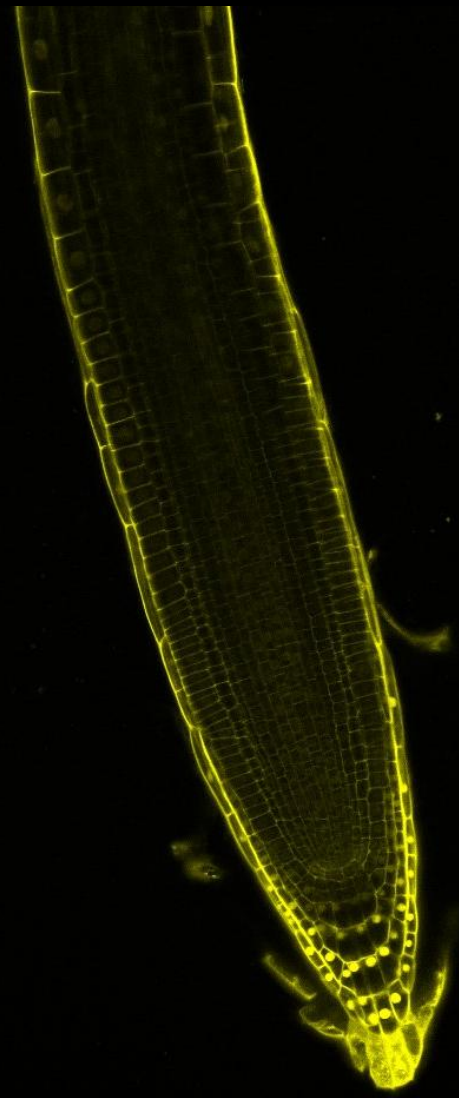
Spatio-temporal patterns of ion and metabolite levels in living cells are important for understanding signal transduction and metabolite flow. The approaches of imaging that use genetically encoded sensors are ideal for detecting such molecular dynamics, which are otherwise difficult to obtain.

Breakthrough in In Vivo Compatible Sensors: Fluorescent Proteins

Origin

Derived from Cnidaria organisms like jellyfish and corals (e.g., Aequorea species).

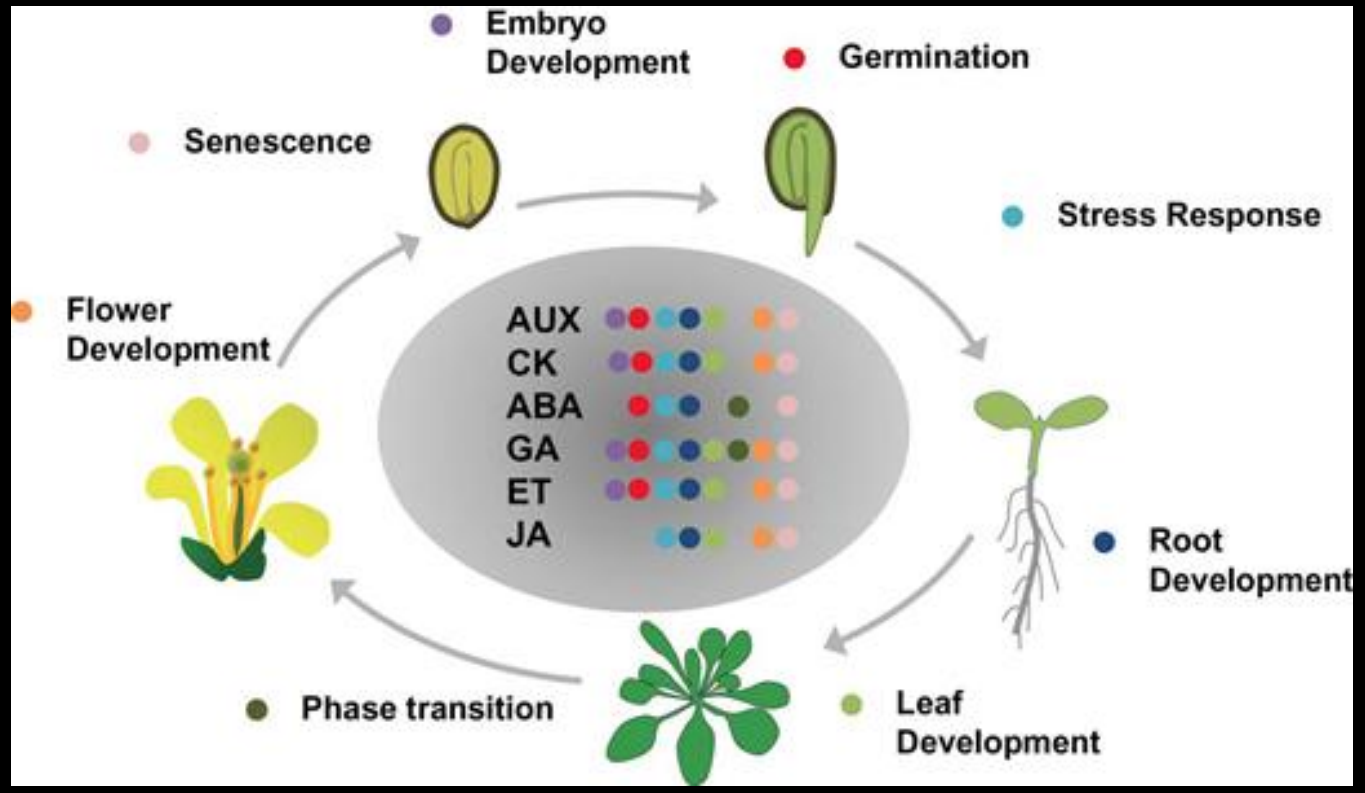
Fluorescent Proteins (FPs) are highly beneficial due to their ability to report cellular events. It has been demonstrated that two FPs acting as a pair of FRET (Förster Resonance Energy Transfer) donors and acceptors can function as reporters of biochemical events with a resolution beyond the limit of optical microscopy.



Biosensors types

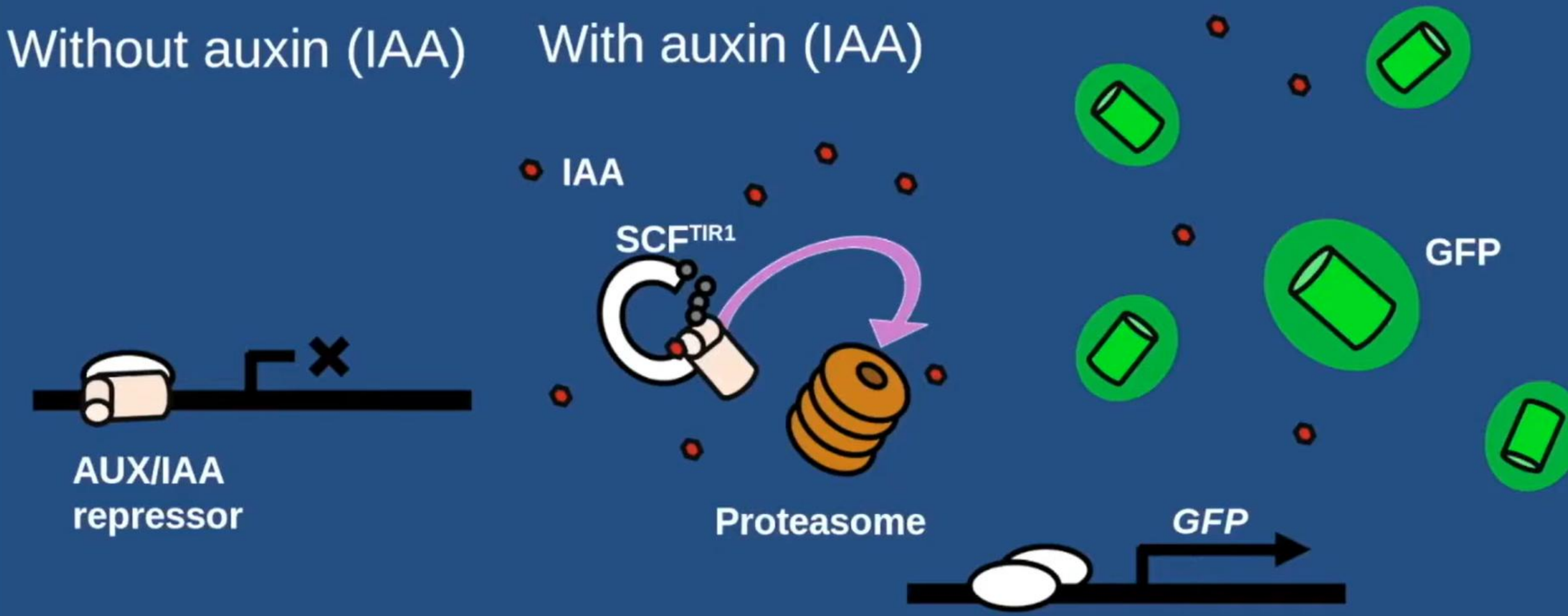
Intensiometri or Ratiometric

- Transcriptional reporters
- Degron reporters
- Direct intrinsic biosensors
- Direct extrinsic biosensors

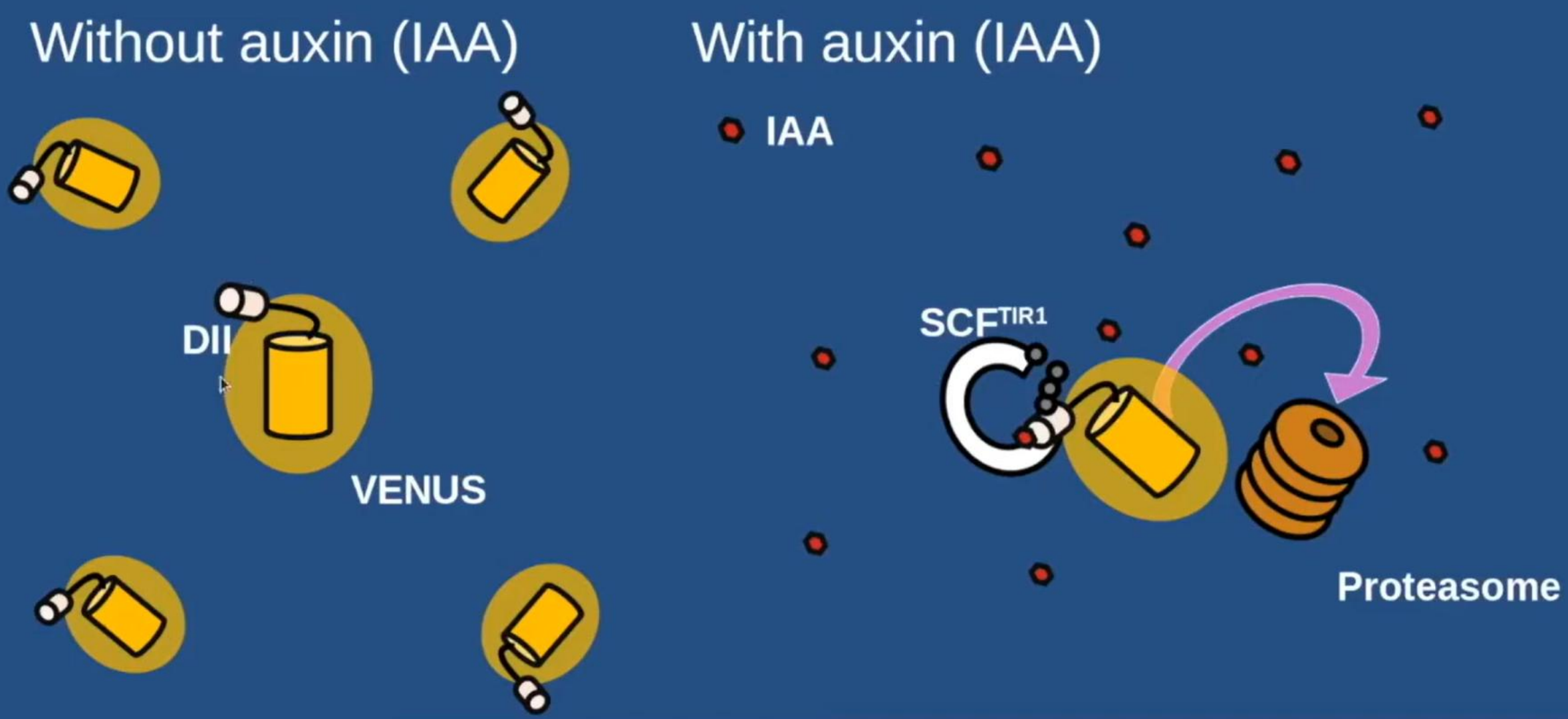


(Curaba *et al.*, 2014)

Transcriptional reporter e.g. DR5:GFP



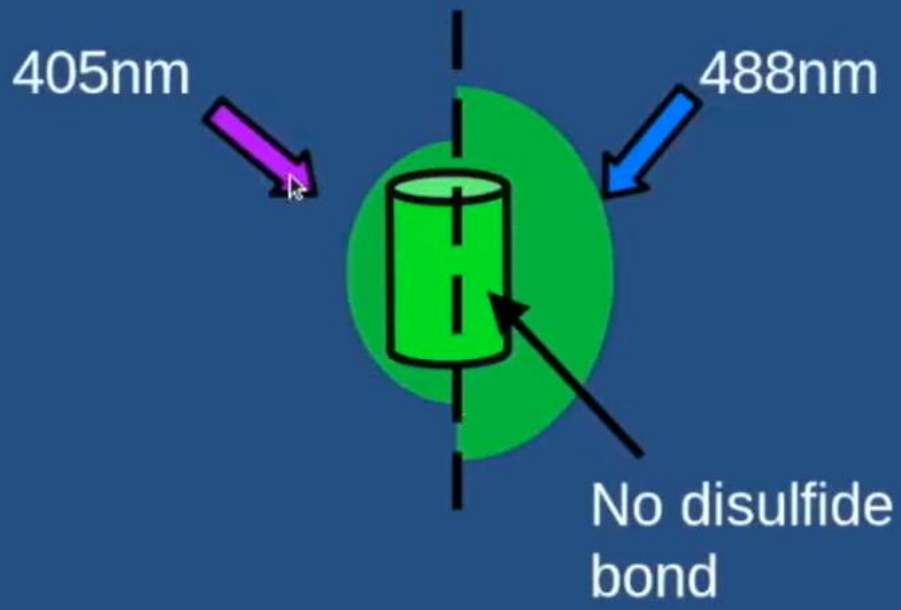
Degron reporters e.g. DII-VENUS



Direct intrinsic biosensors e.g. roGFP

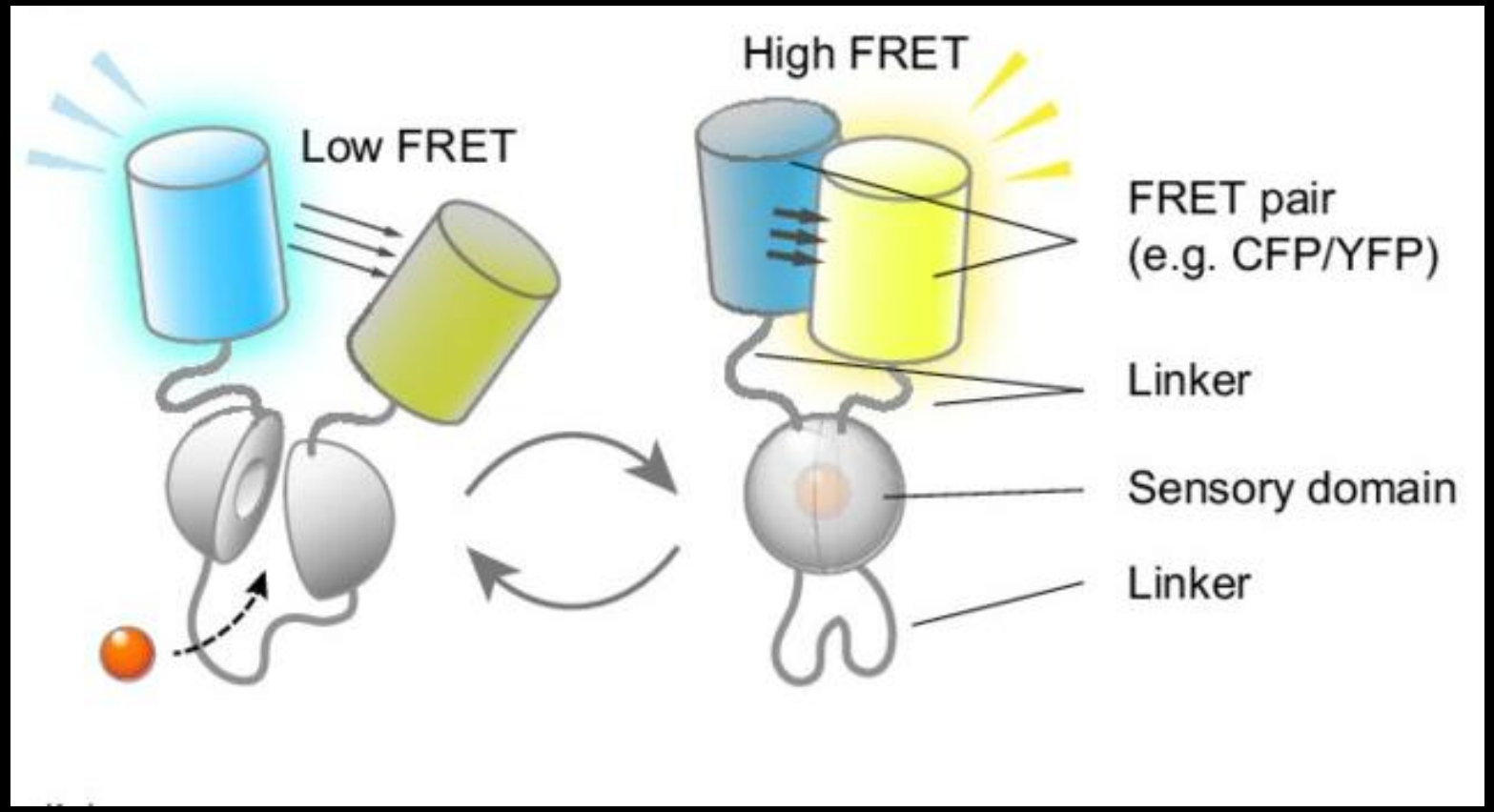
Oxidising environment

Reducing environment



Direct extrinsic Biosensor e.g. FRET based nlsGPS2

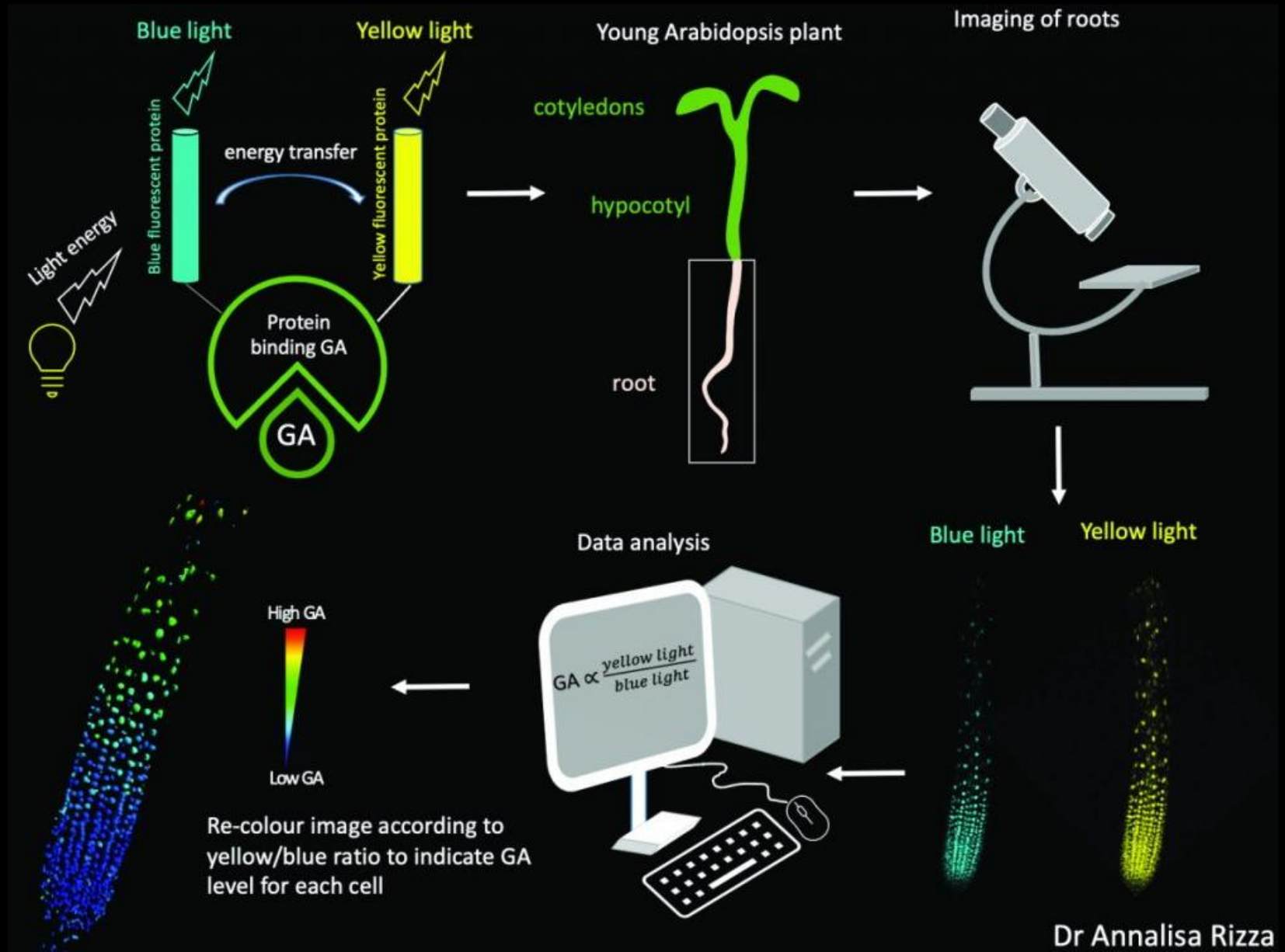
Sensory Domain: Detects changes, triggering FPs' proximity shift.
Linkers: Connect FPs to the sensory domain, allowing movement.
Low FRET: FPs are distant; low energy transfer indicates no event.
High FRET: FPs are close; high energy transfer signals an occurring event.



Direct Biosensor Benefits

- Fast responce to analyte
- Little to no requirement for endogeneous machinery
- Reversibility
- Often ratiometric

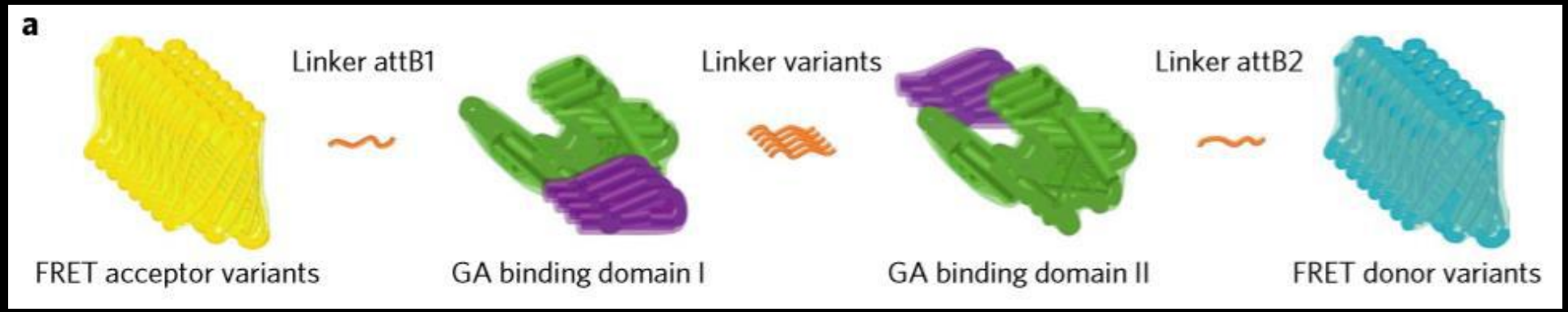
How does the nlsGPS-2 Biosensor work?



Constructing GA FRET Biosensors with Gateway Cloning

Entry clones: clones encoding potential GA-binding domains

Destination Vectors: vectors carrying genes coding for FRET pair variants



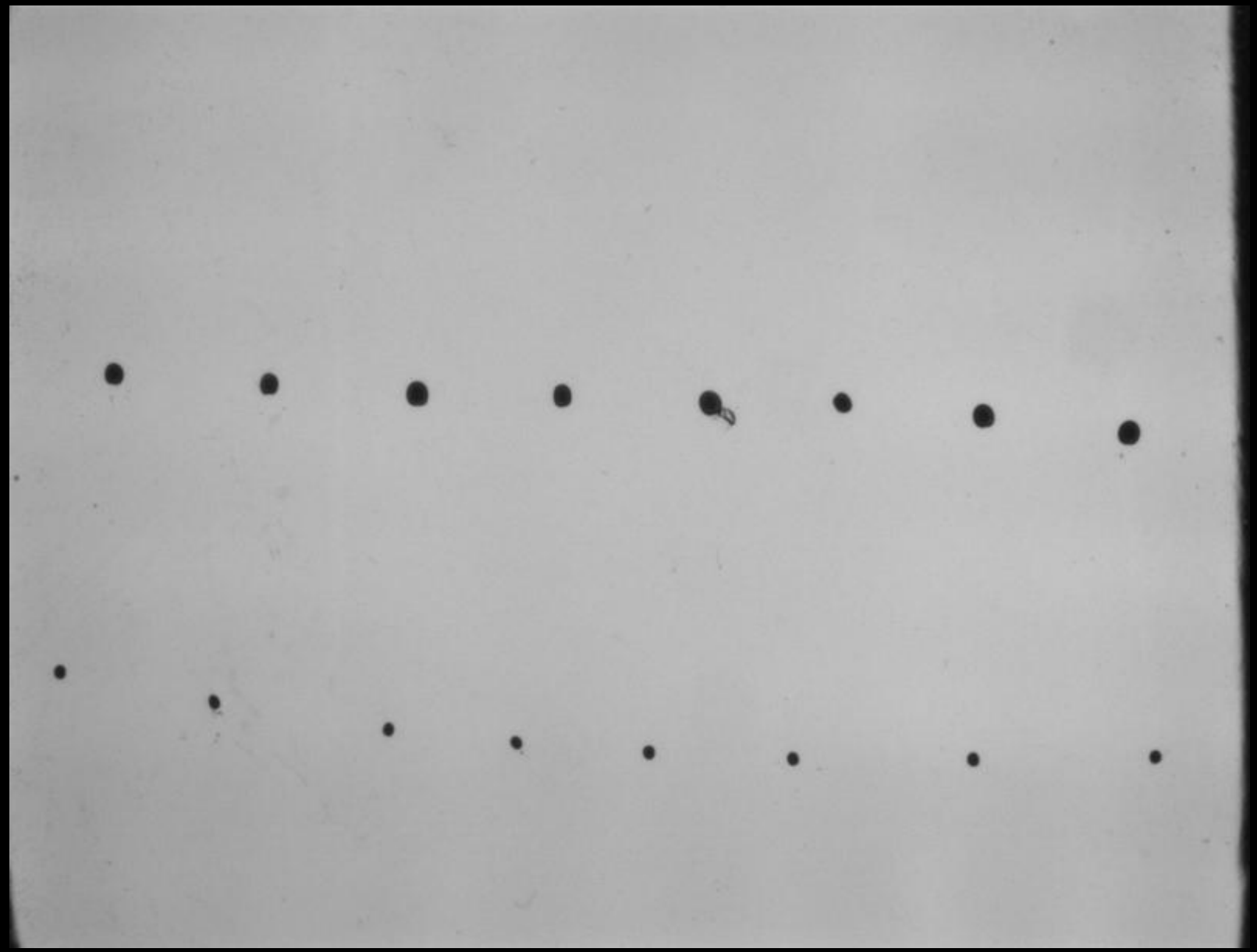
Potential binding domains (GA binding domain I and II): GA receptors, (GID1 A, B or C), linked through a linker of 12 amino acids (L12) at the N- or C-terminus of a truncated DELLA repressor, i.e. GAI or RGA. These potential candidates were recombined with destination vectors expressing the FRET variants of YFP and CFP.

We Built a Biosensor…
Now What?

Germination: Cardamine vs Arabidopsis

Cardamine Hirsuta

Arabidopsis Thaliana

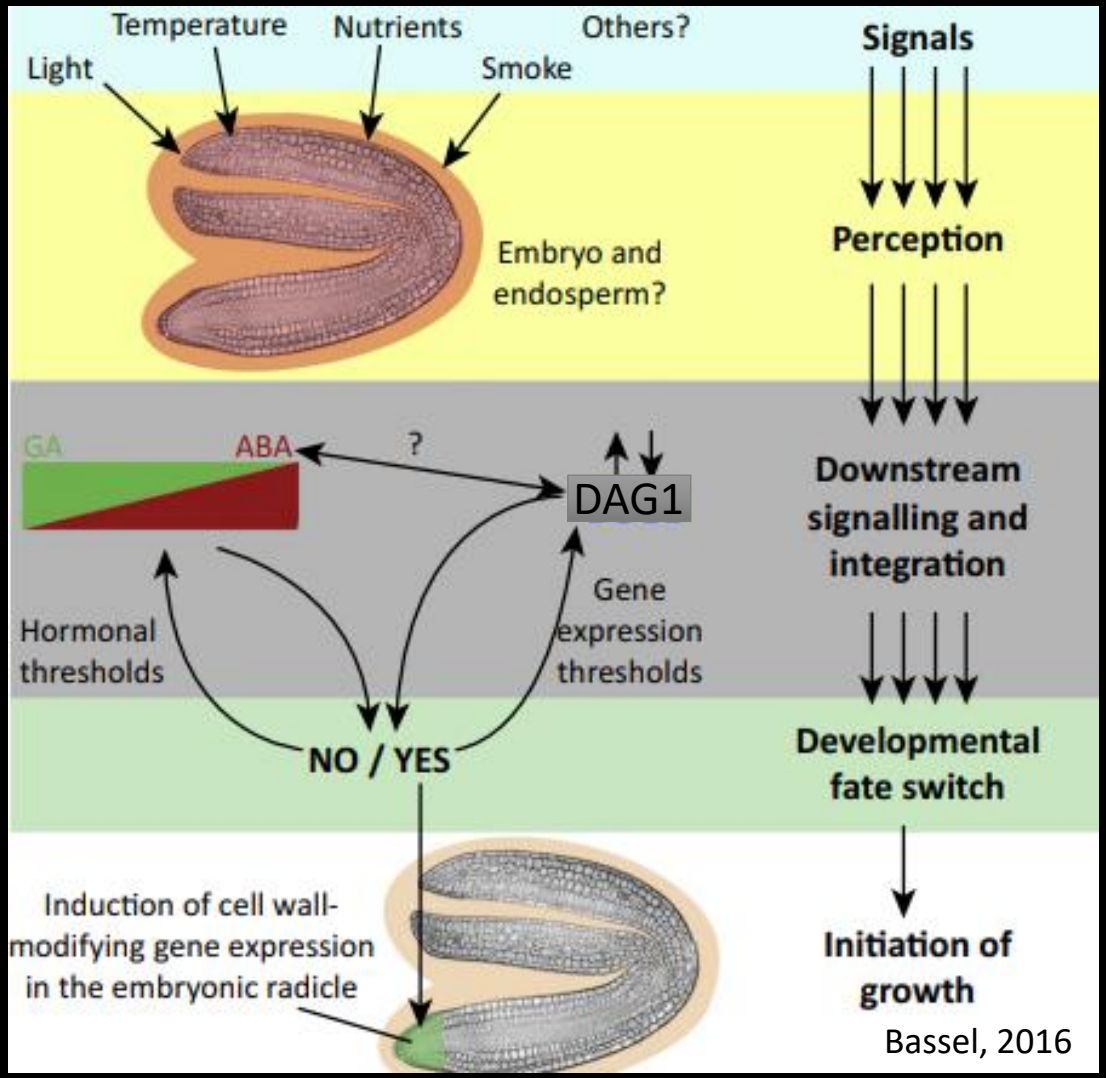


Application of nlsGPS2: Visualizing GA in Germination (Cardamine vs Arabidopsis)

“The hormone behind seed germination”

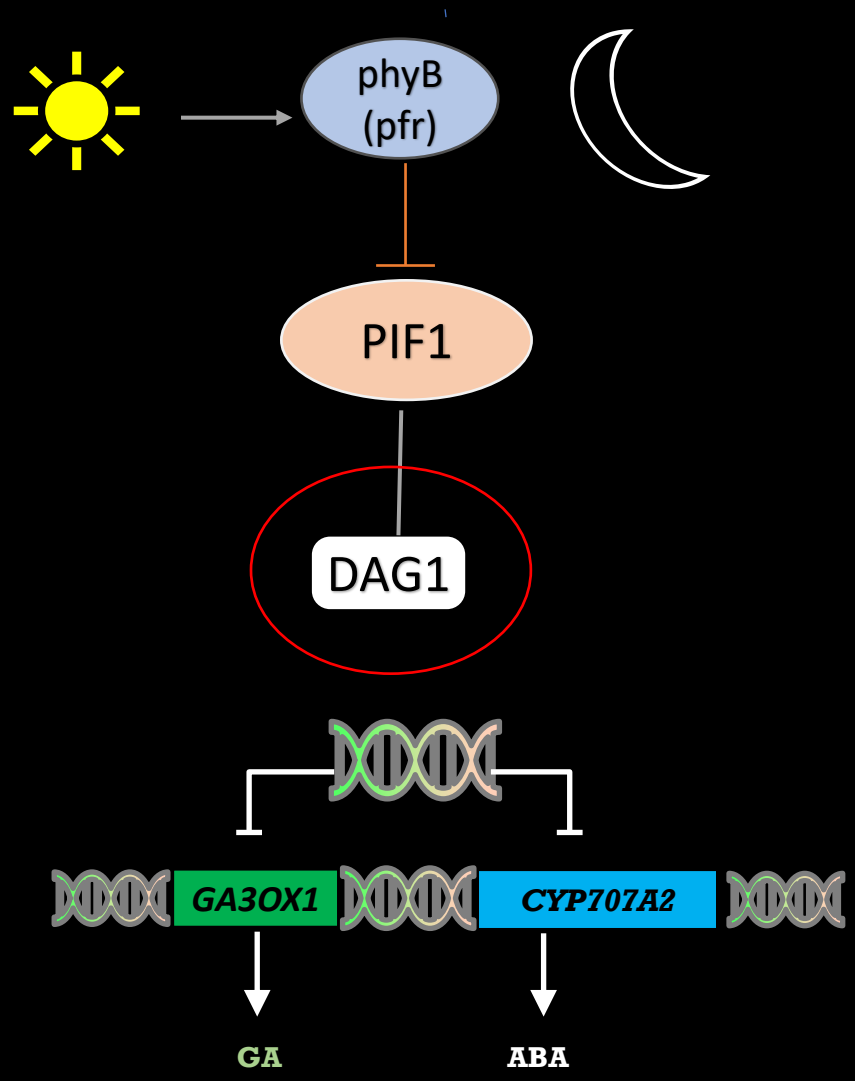
Plants coordinate their growth through hormones – chemical messengers that instruct cells to do certain activities

Let's investigate how one particular plant hormone, gibberellin, affects cells’ behaviour, and what this means for the crops of the future ?

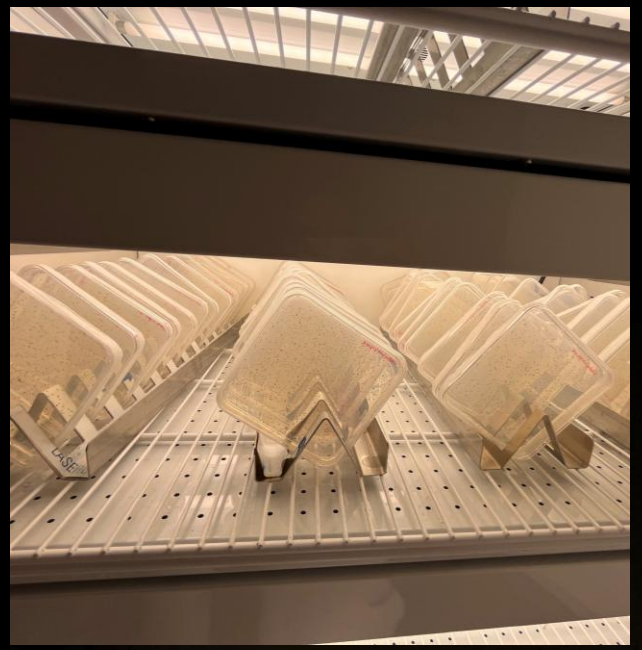


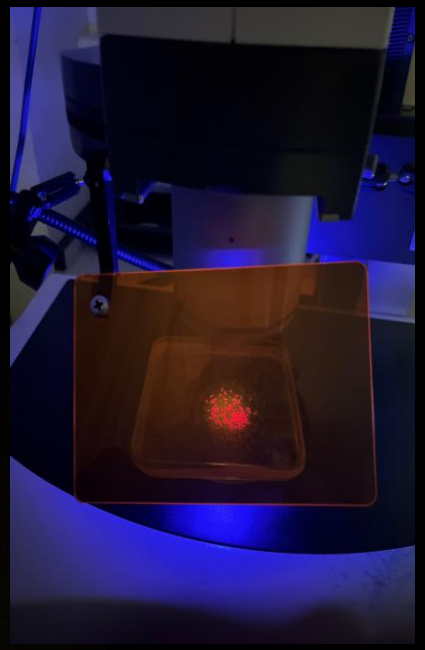
From the top down, multiple signals from the environment are perceived, initiating the process of downstream signalling and signal integration using both hormonal [abscisic acid/gibberellins (ABA/GA) balance] and gene expression (DAG1) thresholds. This integration process collectively acts upon a final irreversible developmental fate switch that, when flipped, initiates the process of embryo growth and growth promoting gene expression principally within the embryo radicle

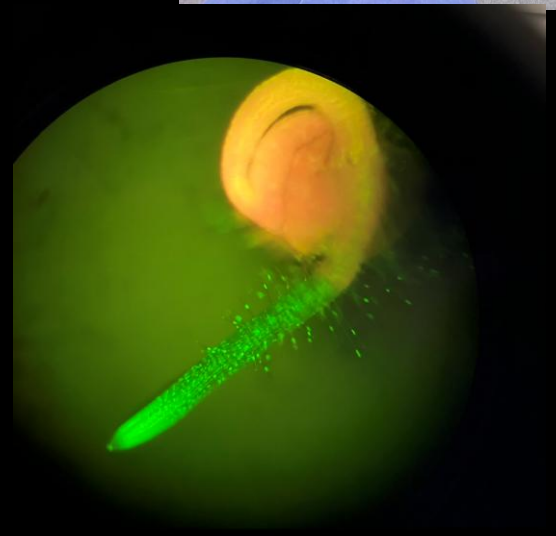
Light Signaling Pathway

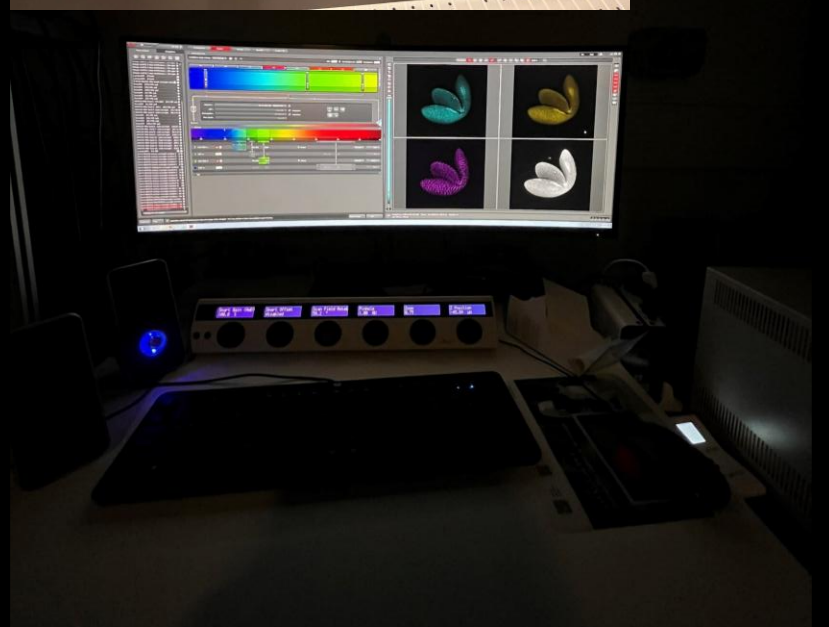


WS and Atdag1 transformation with nlsGPS2 Biosensor

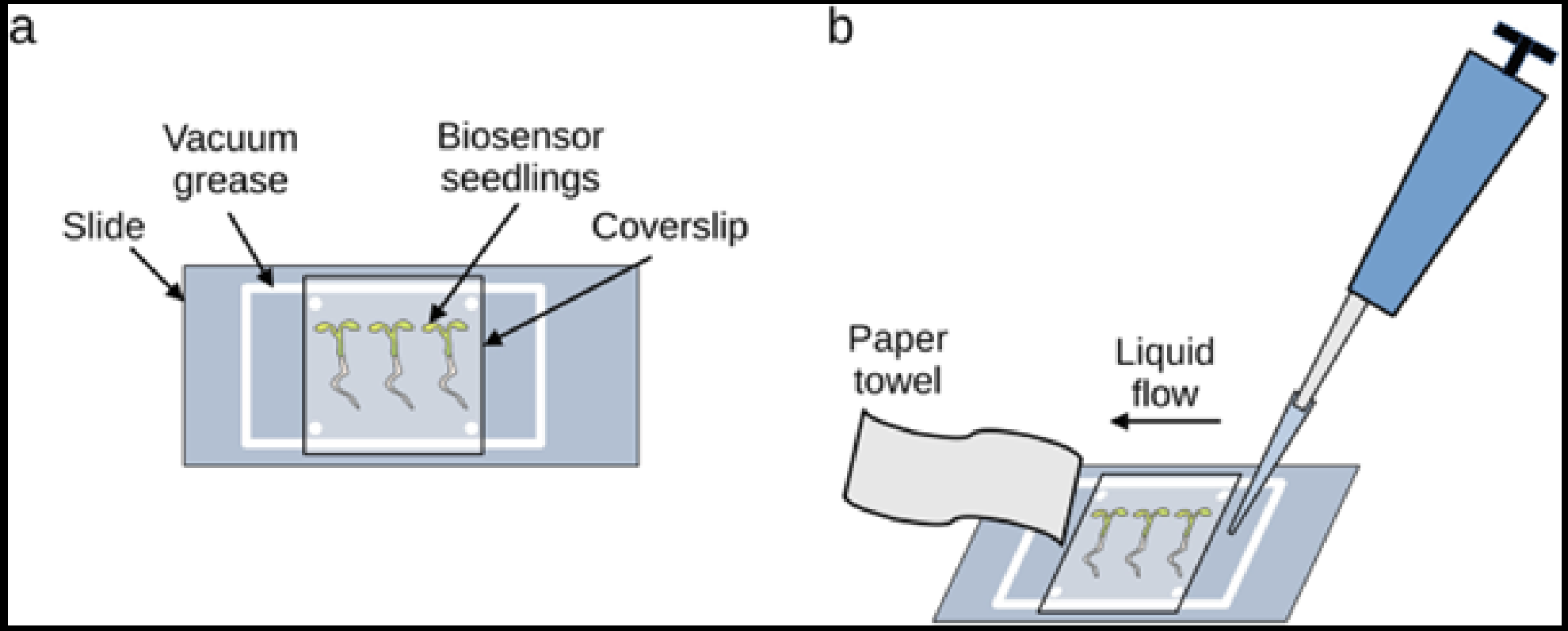








Experimental Design: Confocal Imaging of GA Biosensors



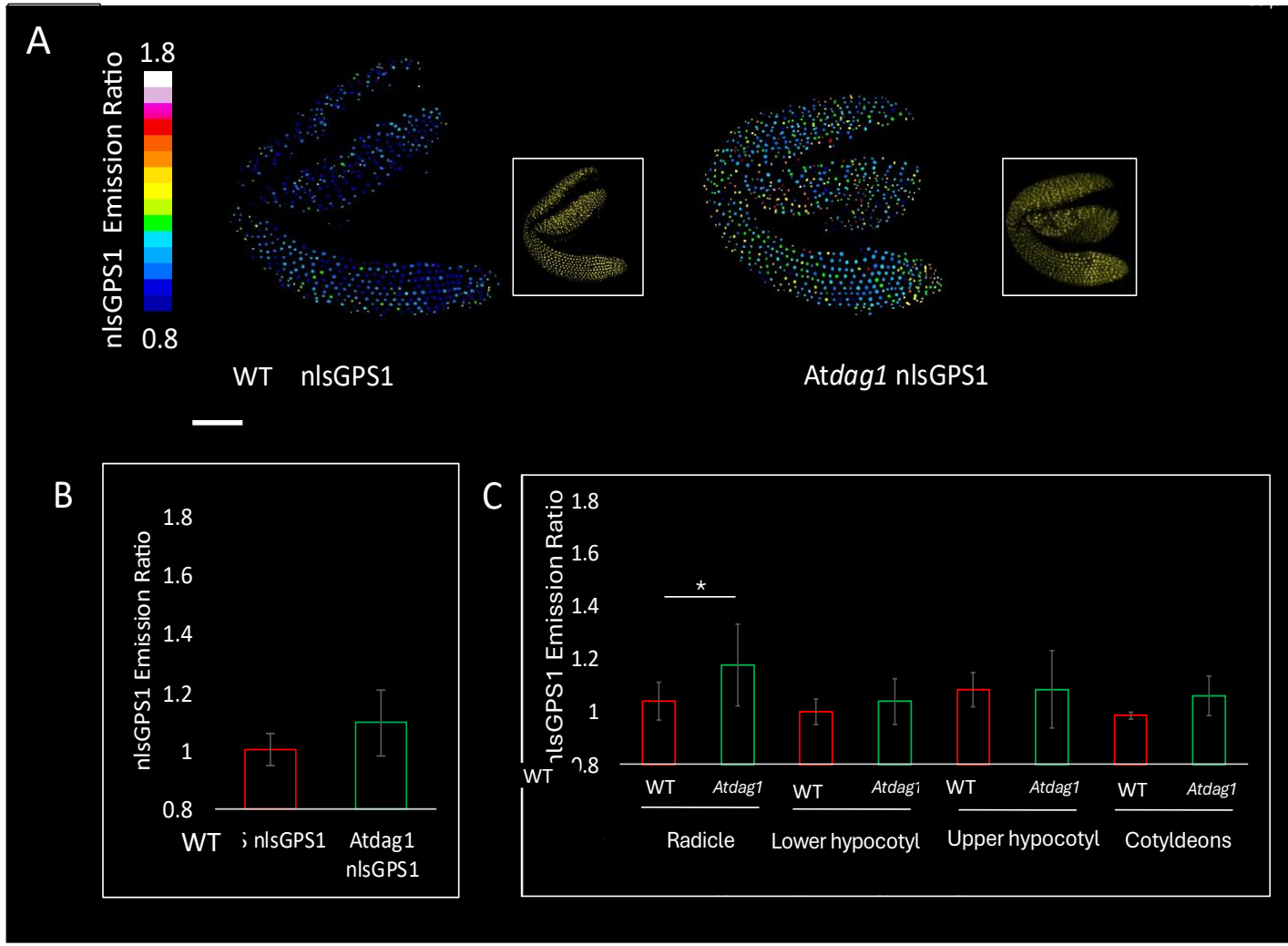
These panels show a schematic representation of the sample preparation for a steady-state experiment

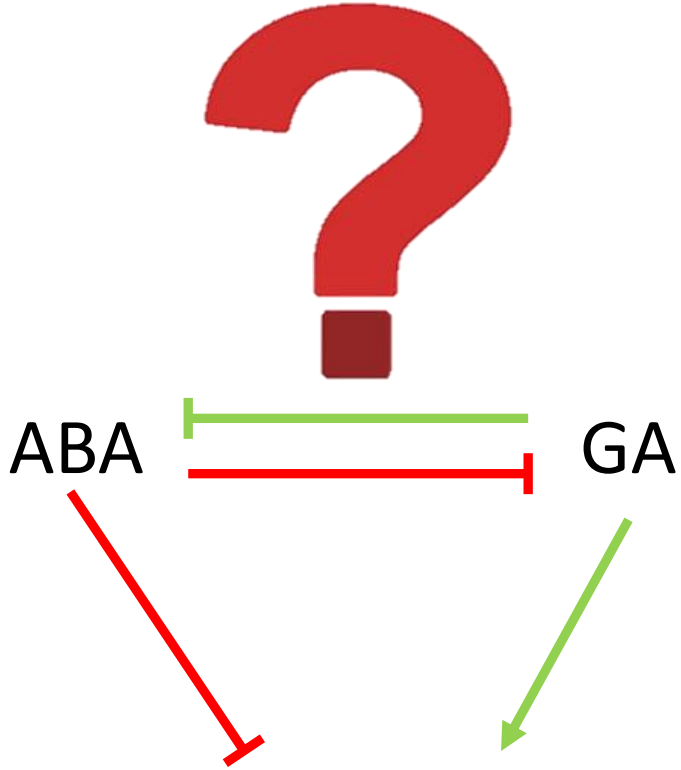
The dag1 mutation leads to an increased GA emission ratio

A) Max Z-projection of emission ratios of nlsGPS1 biosensor in wild-type (left) and *Atdag1* mutant (right) embryos imbibed in the light for 24 h. The pseudocolored scale represents emission ratio values ranging from 0.8 to 1.8. *Atdag1* mutant embryos show higher emission ratios compared to the wild-type.

B) B) Quantification of average emission ratios of nlsGPS1 in wild-type and *Atdag1* embryos. Bars represent the median emission ratios with SD values.

C) Tissue-specific emission ratios of nlsGPS1 in the radicle, lower hypocotyl, upper hypocotyl, and cotyledons. Bars represent the median emission ratios with SD values. *Atdag1* mutants exhibit significantly higher emission ratios in the radicle.



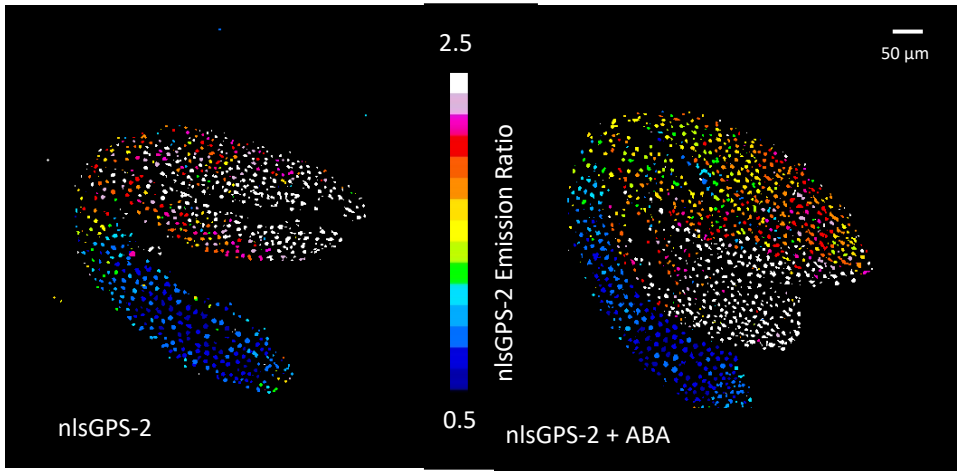


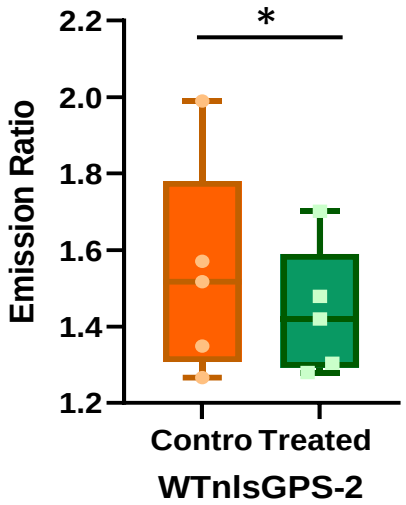
GERMINATION

Interplay between gibberellins (GAs) and abscisic acid (ABA) is crucial for regulating plant processes, including seed germination. While GAs typically promote germination, ABA generally inhibits it. However, recent research has revealed a complex crosstalk between these two hormones, suggesting that their effects are not mutually exclusive.

GA negatively control ABA levels in Arabidopsis embryos

A) Max Z-projection of emission ratios of nlsGPS1 biosensor in wild-type embryos imbibed under 24-hour light conditions. The embryos were treated with 2 µM ABA, and emission ratio imaging was performed to analyze ABA accumulation and response. Significant differences were observed between control and ABA-treated embryos, suggesting that a higher ABA concentration can significantly reduce GA levels. B) Comparison of overall nlsGPS1 biosensor emission ratios in response to 10 µM ABA treatment and untreated control.



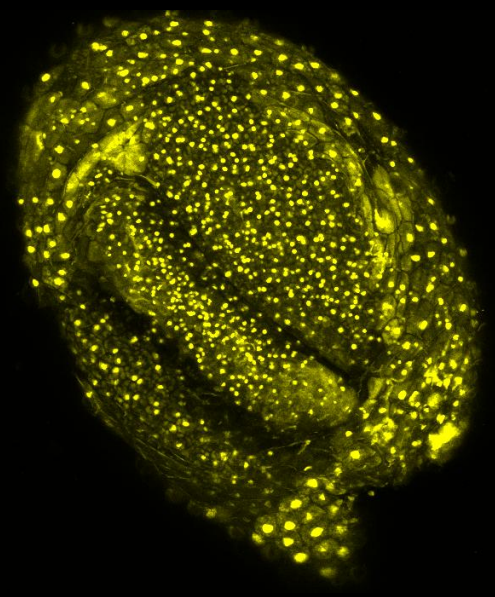


Exploring Cellular Signals in Endosperm: Understanding True Signals

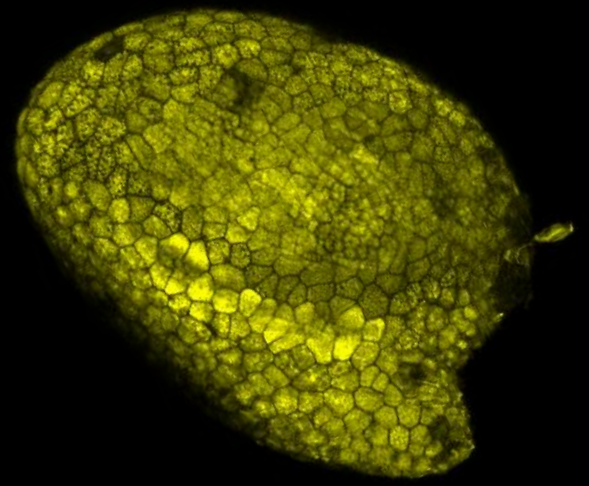
Analyzed signals in endosperm of Col-0 seeds with sensor.
Compared against endosperm of seed without sensor.

Findings:

- Observed potential signals in the endosperm of seeds transformed with Biosensor
- No signals detected in seeds without sensor, suggesting observed signal in first sample likely due to true signal.



Endosperm with biosensor



Col-0 Endosperm without biosensor

FINDINGS AND APPLICATIONS

Live hormone Imaging: Genetically encoded biosensors (e.g. FRET-based GPS1/2) now enable real-time, cellular-level visualization of hormone distribution in vivo.

Spatial GA Gradients: These tools reveal distinct GA gradients and localization patterns linked to growth. High GA concentrations in rapidly elongating cells (roots, dark-grown hypocotyls) correlate with greater cell expansion, whereas GA is locally depleted during light-triggered developmental transitions (photomorphogenesis).

Seed Germination Insights: Applied to seed biology, GA biosensors illuminate hormone dynamics during germination. Light cues induce GA accumulation in the embryonic root (radicle) to break dormancy.

Agricultural Relevance: GA sensor findings inform crop improvement by guiding fine-tuning of GA levels for higher yield and stress resilience. The goal is to minimize trade-offs e.g. semi-dwarf, lodging-resistant crops that maintain grain yield: moderate GA reduction can improve drought tolerance and lodging resistance but excessive GA suppression can impair reproductive development (shorter GA-deficient rice failed to form grains properly).