Rising to the Challenge: Developing Biosensors to Study Nitrogen Transport in Plants

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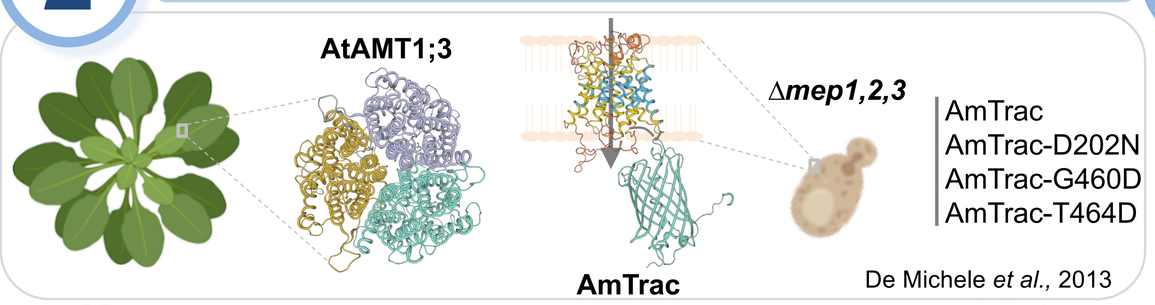
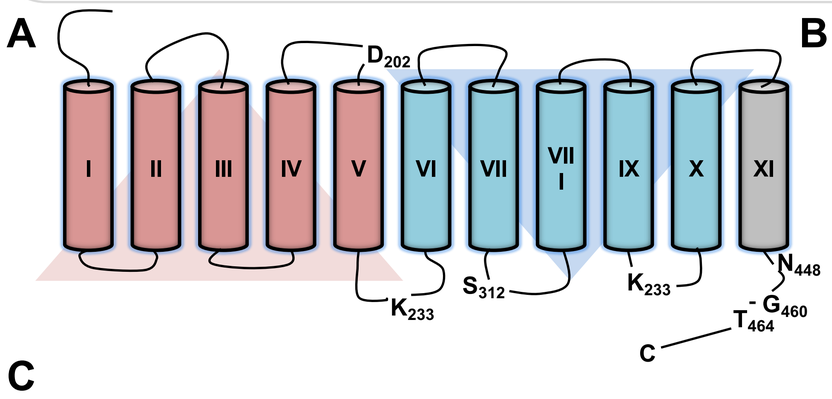
1 INTRODUCTION

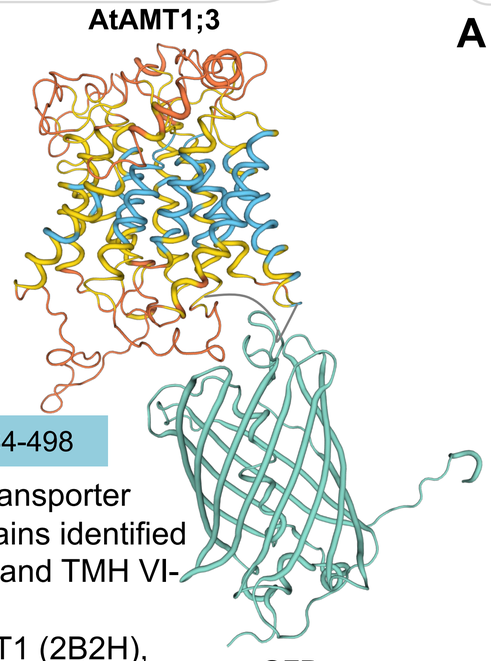
Nitrogen (N) is vital for plant development and growth, leading to its widespread use in agricultural crops as fertilizers. Unfortunately, the excessive N from fertilizers often leaches into groundwater, contaminating drinking water sources and posing environmental and health risks. Understanding nitrogen compound assimilation, transport, and biosynthesis is critical for enhancing plant growth. However, many biological and metabolic processes in plants at cellular and subcellular levels remain unknown due to limited real-time monitoring tools. The assimilation of inorganic and organic nitrogen involves various transporter systems in plant cell membranes, but their distribution and functions across different cellular types and compartments are still unclear.

To address this, we propose utilizing dual ratiometric biosensors equipped with fluorescent proteins in subcellular compartments. Building on previous glutamate sensor research in plants (Castro-Rodriguez et al., 2021), we identified promising candidates like the ammonium transporter PpAMT1.3 (Castro-Rodriguez et al., 2016) and the amino acid permease PpAAP1 from the conifer *Pinus pinaster* (Llebres et al., 2022). Implementing these biosensors could shed light on nitrogen dynamics in plants and aid in improving plant growth and development.

2 Design of biosensors in membrane proteins: AmTracs





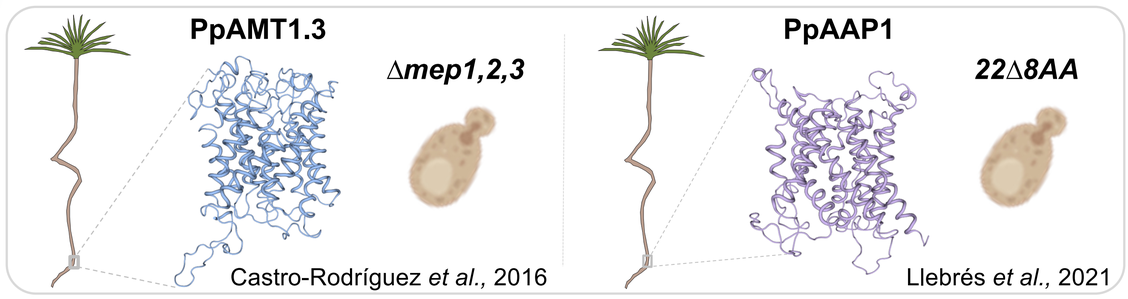


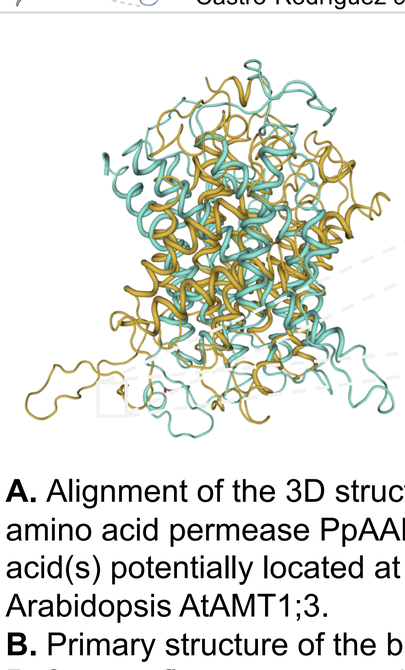
C

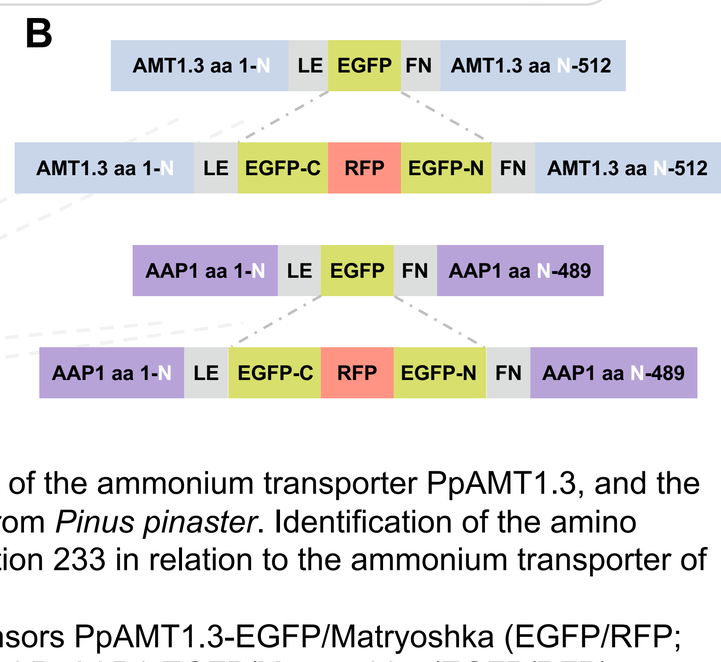
AMT1;3 aa 1-233	LE	cpGFP	FN	AMT1;3 aa 234-498

A. Representation of the 2D topology of the ammonium transporter from Arabidopsis AtAMT1;3. Eleven transmembrane domains identified and organized in a pseudo-symmetric structure (TMH I-V and TMH VI-X).
B. Three-dimensional model of the AmTrac sensor; AfAMT1 (2B2H), and cpGFP (circular permutated Green Fluorescent Protein) (3evp). Insertion position corresponding to amino acid 233 between domains V and VI.
C. Primary structure of the biosensor: AmTrac (De Michele et al., 2013).

3 Nitrogen transporters in forest plants of interest: Candidates

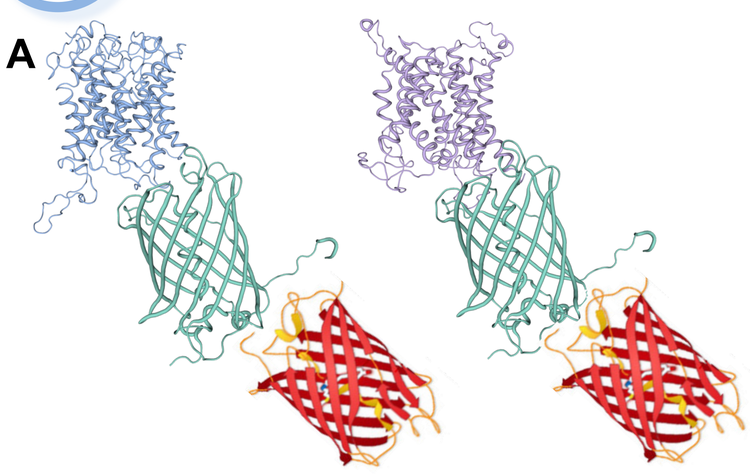






A. Alignment of the 3D structures of the ammonium transporter PpAMT1.3, and the amino acid permease PpAAP1, from *Pinus pinaster*. Identification of the amino acid(s) potentially located at position 233 in relation to the ammonium transporter of Arabidopsis AtAMT1;3.
B. Primary structure of the biosensors PpAMT1.3-EGFP/Matryoshka (EGFP/RFP; Reference fluorescent protein) and PpAAP1-EGFP/Matryoshka (EGFP/RFP).

4 Designing of N Transporter Biosensors

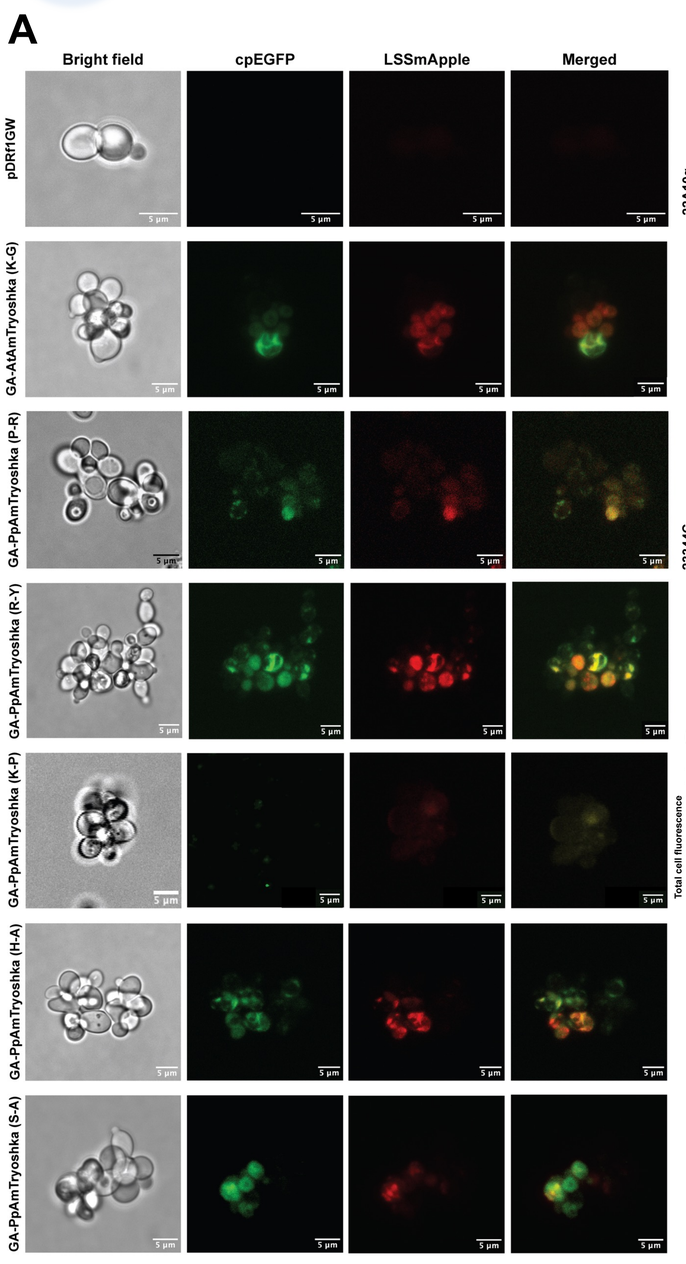


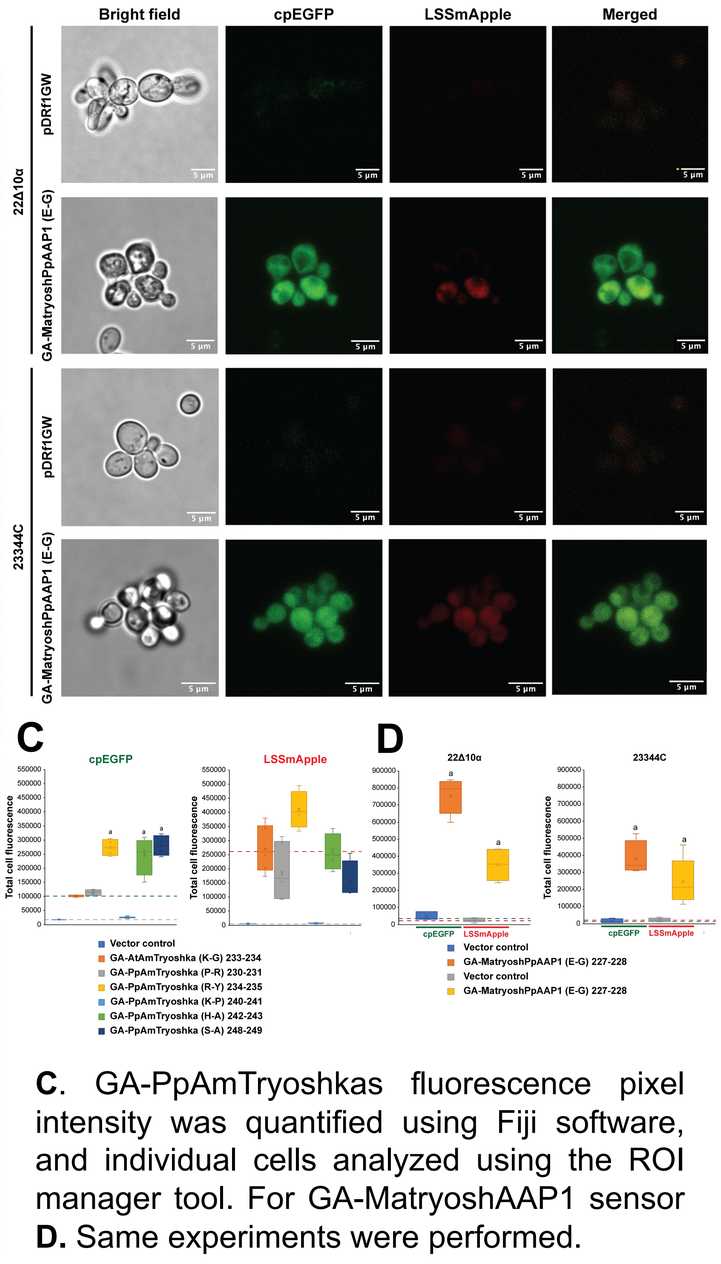
GA-PpAmTryoshkas **GA-MatryoshAAP1**

AMT1.3 aa 1-N1	LE	EGFP-C	RFP	EGFP-N	FN	AMT1.3 aa N2-512
AMT1.3 aa 1-N3	LE	EGFP-C	RFP	EGFP-N	FN	AMT1.3 aa N4-512
AMT1.3 aa 1-N5	LE	EGFP-C	RFP	EGFP-N	FN	AMT1.3 aa N6-512
AMT1.3 aa 1-N7	LE	EGFP-C	RFP	EGFP-N	FN	AMT1.3 aa N8-512
AMT1.3 aa 1-N9	LE	EGFP-C	RFP	EGFP-N	FN	AMT1.3 aa N10-512
AAP1 aa 1-N1	LE	EGFP-C	RFP	EGFP-N	FN	AAP1 aa N2-489

A. Three-dimensional model of the sensors PpAMT1.3-Matryoshka and PpAAP1-Matryoshka; composed of the reference fluorescent protein embedded between the C and N terminals of the fluorescent protein GFP (EGFP).
B. Primary structure of the biosensors PpAMT1.3-Matryoshka (EGFP/RFP) and PpAAP1-Matryoshka (EGFP/RFP) using 5 and 1 alternative positions, respectively, close to the insertion site in relation to the membrane biosensor, AmTrac.

5 Proof of concept: N Transporter Biosensors in Yeast





C. GA-PpAmTryoshkas fluorescence pixel intensity was quantified using Fiji software, and individual cells analyzed using the ROI manager tool. For GA-MatryoshAAP1 sensor **D.** Same experiments were performed.

A. GA-PpAmTryoshkas sensors were expressed in yeast cells, and confocal sections were obtained using confocal laser scanning microscope (SP8 Stellaris; Leica, Germany). Fluorescence excitation was achieved using a 488 nm laser and emission intensities were using a 525/50 nm emission filter for GFP, 617/73 nm emission filter for LSSmApple. For GA-MatryoshAAP1 sensor **B.** Same experiments were performed.

6 CONCLUSIONS

GA-PpAmTryoshkas and GA-MatryoshAAP1 sensors are the first prototypes of nitrogen transport biosensors in conifers, designed for real-time monitoring of nitrogen acquisition in forest plants. Experiments in plant systems are necessary to validate the functionality of these sensors.

7 References

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