



EPSRC & BBSRC Centre for Doctoral

Training in Synthetic Biology



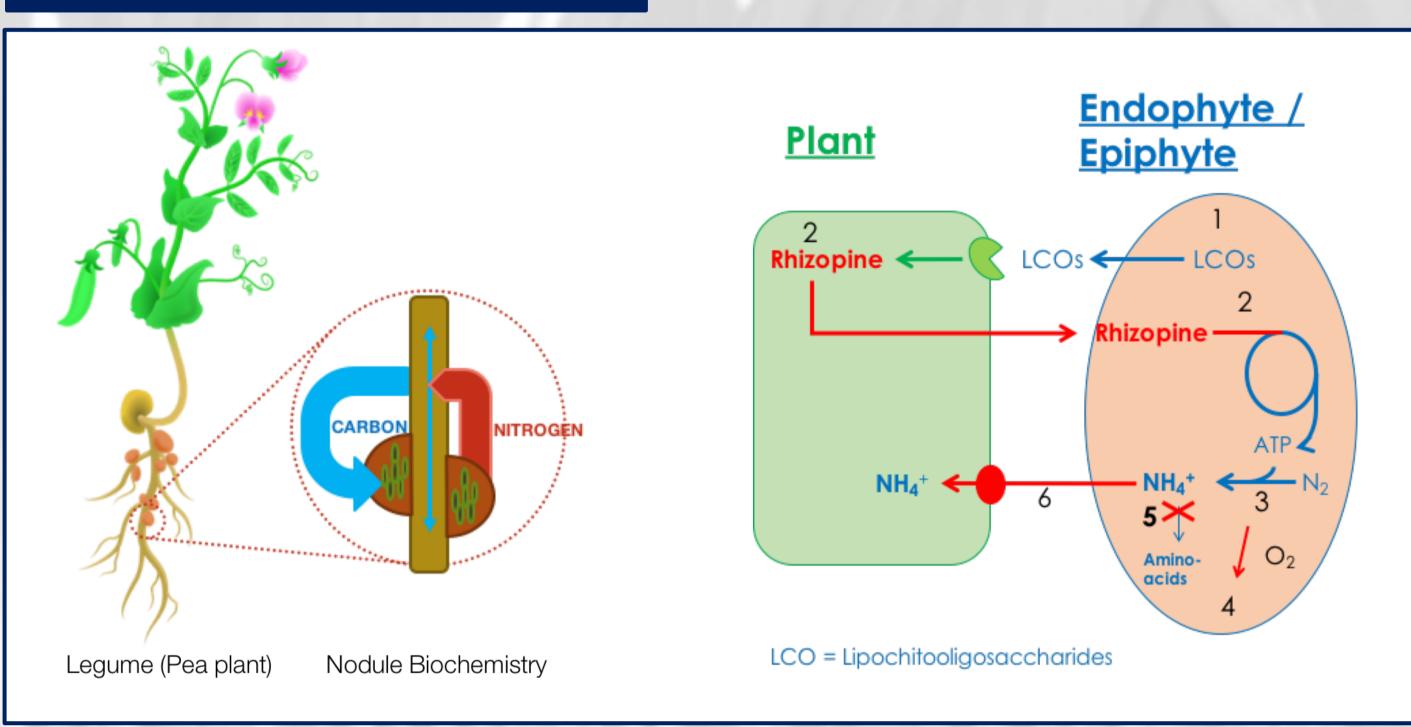
Constructing a Rhizosphere-Specific Genetic Switch:

Engineering Rhizobia as a Synthetic Biology Platform

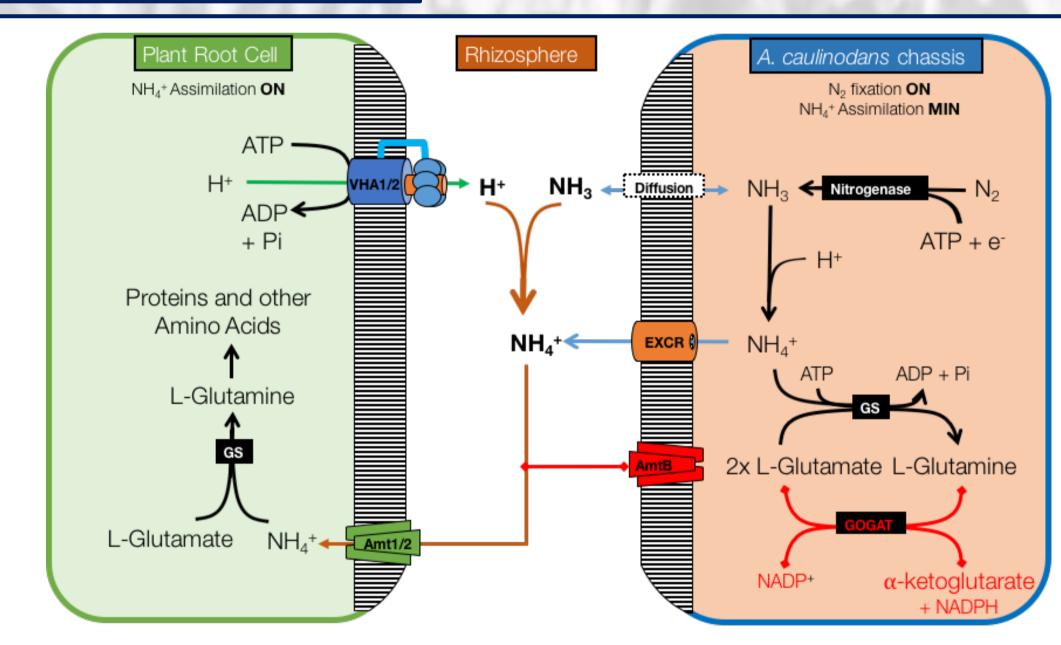
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In this project we aim to contribute to the engineering of bio fertilisers that could alleviate the use of inorganic fertilisers and serve as an economically viable alternative in developing countries. *Azorhizobium caulinodans* and *Azospirillum brasilense*, two diazotrophic nitrogen-fixing and known plant endophytes are used as chassis for genetic manipulation. Genetic switch circuitry are used to control expression of core enzymes in the nitrogen assimilation pathway of the organism. Using rhizosphere-specific inducers, the chassis will perform a phenotypic switch from a competitive to ammonia-secreting phenotype. This will allow a rhizosphere specific phenotypic switch from a fast-growing competitive state to a plant beneficial ammonia secreting state.

NATURAL AND SYNTHETIC SYMBIOSES

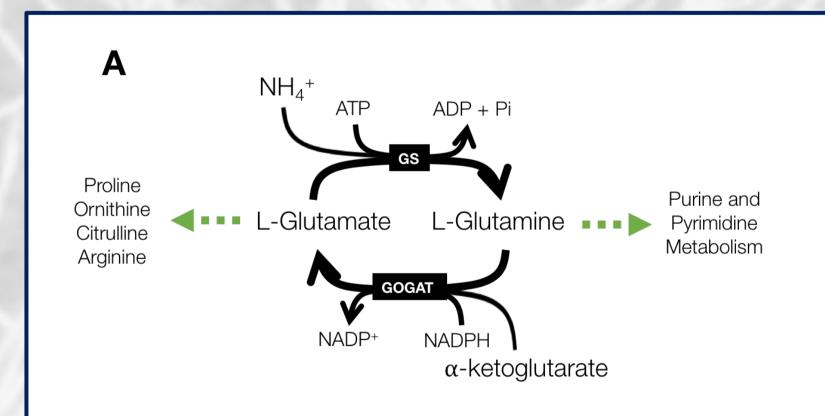


AMMONIA TRANSFER MECHANISM



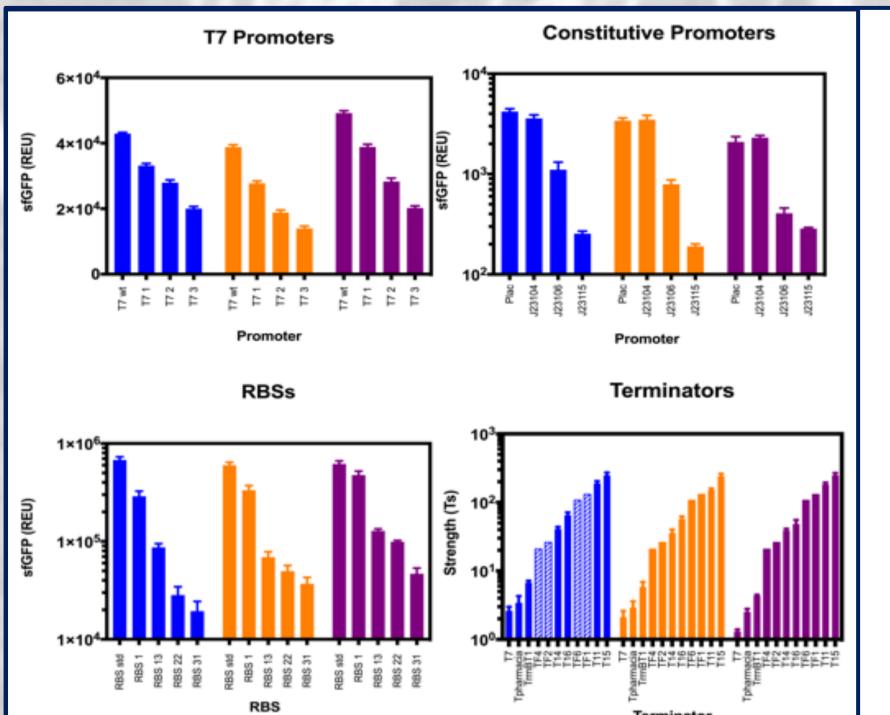
 Ammonia produced from fixed atmospheric nitrogen is diverted from assimilation and biosynthesis. Instead the concentration builds within the cell and the ammonia is excreted. Re-uptake by AmtB inhibited.

TARGETS FOR CONTROL:



- Glutamine synthetase (GlnA)
- Glutamine oxoglutarate aminotransferase (GltBD)
- Ammonia channel uptake protein (AmtB)

DNA PART CHARACTERISATION

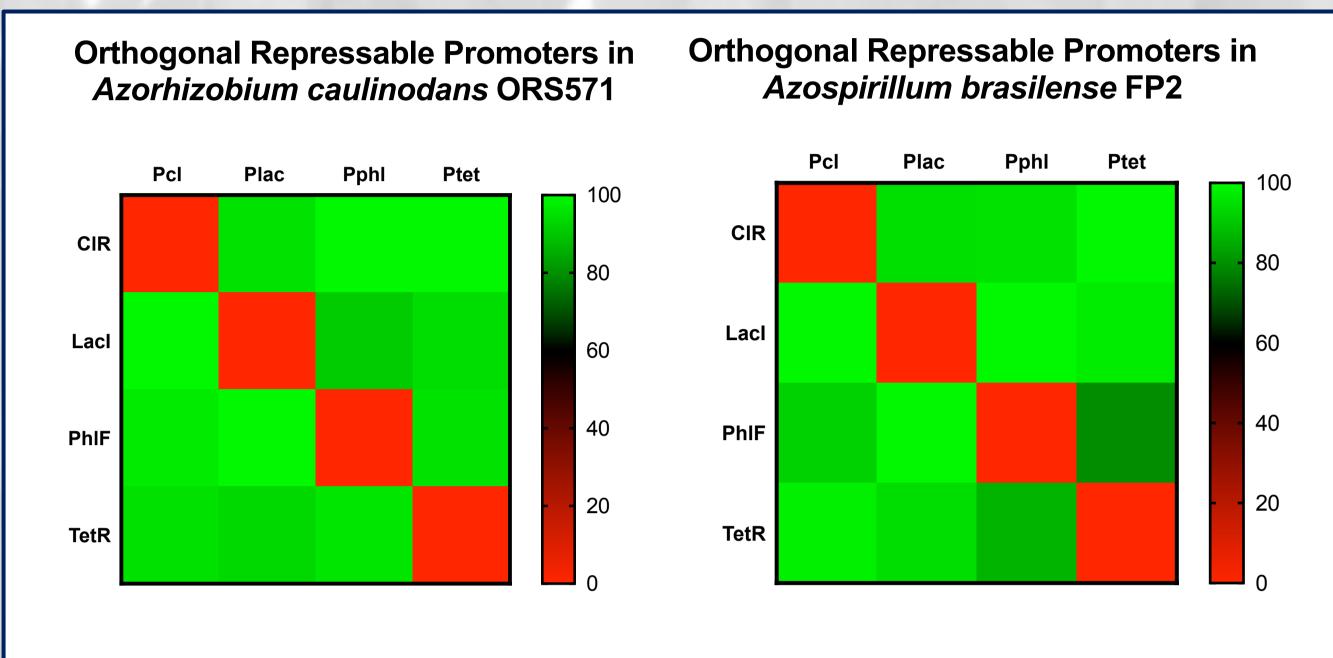


- Orthogonal promoters (T7 RNAP driven), constitutive (sigma70) ribosome binding sites (RBSs) and terminators.
- DNA part suites built out with different levels of transcription, translation and termination.
- Characteristics of DNA parts carry across multiple *Rhizobia* species.

REFERENCES:Brophy, JA & Voigt, CA (2014) Principles of Genetic Circuit Design. *Nat. Meth.* 11: 508-511

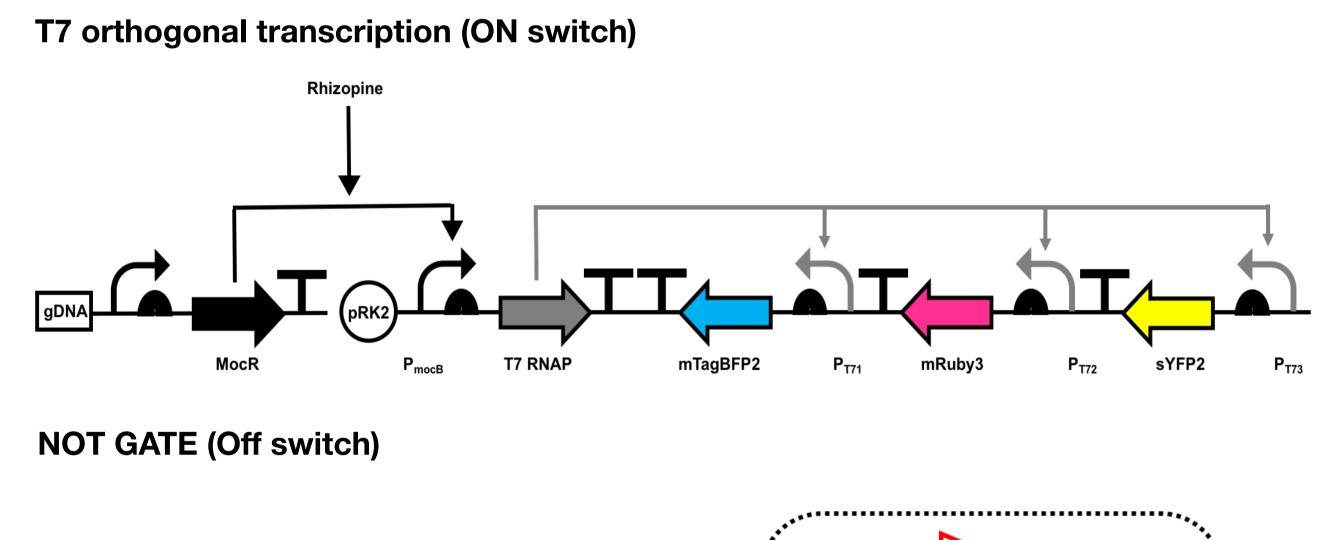
Goppalaswamy G, Kannaiyan S, O'Callaghan KJ, Davey MR and Cocking EC (2000) The xylem of rice (Oryza sativa) is colonized by Azorhizobium caulinodans. Proc Biol Sci 267(1439): 103–107 Geddes BA, Ryu MH, Mus F, Garcia Costas A, Peters JW, Voigt CA, Poole P (2015) Use of plant colonizing bacteria as chassis for transfer of N₂-fixation to cereals. Curr Opin Biotechnol 32: 216-222 Setten L, Soto G, Mozzicafreddo M, Fox AR, Lisi C, Cuccioloni M, Angeletti M, Pagano E, Díaz-Paleo A, Ayub ND (2013) Engineering Pseudomonas protegens Pf-5 for nitrogen fixation and its application to improve plant growth under nitrogen-deficient conditions. PLoS One 8(5

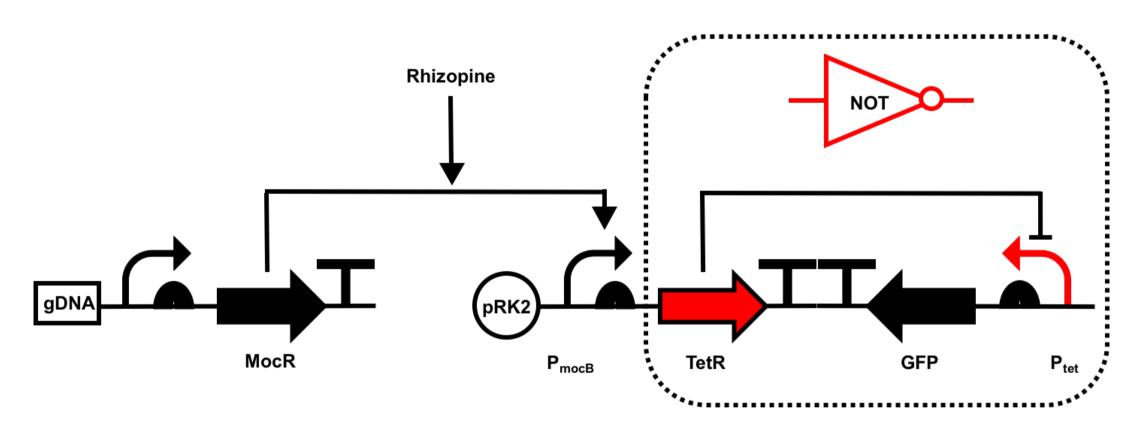
TRANSCRIPTIONAL REPRESSORS

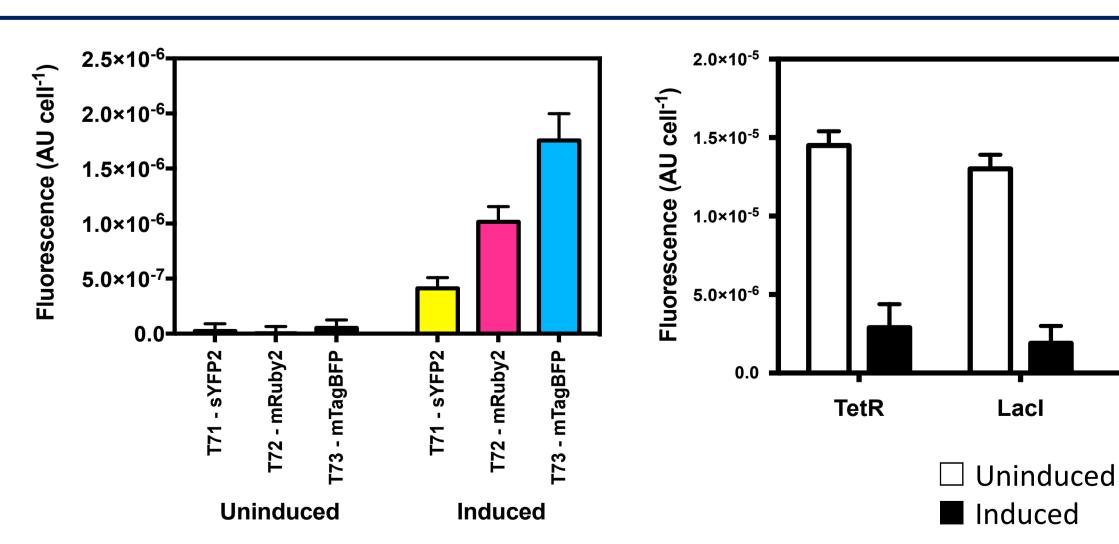


- Analysis of orthogonal promoters and transcriptional repressor pairs allows for complex genetic circuitry and logic functions to be built.
- No significant cross-talk interaction between 4 different transcriptional repressors.

GENETIC SWITCH ARCHITECTURES







• T7 orthogonal transcription allows for simultaneous multigene expression with tuning of expression levels of each gene.

PhIF

 NOT gate function allows for the repression of multiple genes and the construction of more complex logic functions such as AND gates.

FUTURE DIRECTIONS

- Test circuitry with phenotypically relevant genes (GlnA, AmtB, GltBD).
- Tune genetic circuitry for maximum ammonia release per cell with minimum affect on growth.