# Measurement of a possible signal for nitrogen starvation in a cyanobacterium-plant symbiosis

OUTLINE

I. Introduction
   A. We rely heavily on nitrogenous fertilizers, and this causes big problems.
      Cost and ecology.
   B. Legumes don't need nitrogenous fertilizers, from symbioses with rhizobia.
      But they are minor players.
   C. How to extend N-fixation to major crops?
      Rhizobia are specific to legumes. *Nostoc* is a generalist, a more likely candidate.
   D. The host plant modifies *Nostoc* perception of starvation.
      Response by *Nostoc* to ammonia differs in free-living and symbiotic states
   E. α-ketoglutarate may play key role in perception of starvation.
   F. Li et al (2003) test of α-ketoglutarate as signal for N-starvation
      Introduction of α-ketoglutarate into *Nostoc* causes misperception of starvation.
   G. Maybe plants manipulate α-ketoglutarate in *Nostoc* to simulate starvation?

II. Experiment
   A. Summary of experiment: Measure α-ketoglutarate in *Nostoc* with biosensor
   B. FRET biosensors as sensitive detectors of metabolites
      Principle behind method
   C. Example of FRET use (Hires et al, 2008)
      Show spectra. Show biological utility (glutamate biosensor measures time course of neurotransmitter release)
   D. Introduce biosensors used in experiment.
      No α-ketoglutarate biosensor! Use glutamate and glutamine biosensors instead.
   E. Introduction of biosensors into *Nostoc* and *Nostoc* into plant
   F. Measurement of fluorescence

III. Discussion
   A. Best possible results.
      But even this isn't good enough: glutamate/glutamine isn't α-ketoglutarate
   B. Discussion of α-ketoglutarate biosensor
      Choice of base protein. Very time consuming.
   C. Problems with glutamate and glutamine biosensors
      - endogenous fluorescence
      - wrong sensitivity
      - activation by aspartate
      - dependence on ionic strength
   D. Inspirational final words