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

1. Introduction
2. Green Revolution saves lives at high cost
   1. Green Revolution – Plants that can exploit higher nitrogenous fertilizer
   2. Great cost: making ammonia expensive
   3. Great cost: Ecological damage
3. Biological nitrogen fixation: Good but limited
   1. Legumes don't need nitrogenous fertilizers, fix N2
   2. Avoids costs of nitrogenous fertilizer
   3. N-fixation from symbioses with rhizobia
   4. N-fixation limited to legumes (**Figure: world agriculture**)
4. How to extend N-fixation to major crops?
   1. Extend rhizobial to cereals? Rhizobia too specific
   2. Alternative: Cyanobacterium *Nostoc* is a generalist
   3. *Nostoc* achieves generality through independence, heterocysts
5. N-fixation by *Nostoc* different inside plant vs outside
   1. Free-living *Nostoc* hordes N
   2. Symbiotic *Nostoc* shares N
   3. Symbiotic *Nostoc* fixes more N
   4. Host plant modifies *Nostoc’s* perception of starvation?
6. Possible key to inside vs outside: α-ketoglutarate
   1. α-ketoglutarate central to N-metabolism (**Figure: pathway**)
   2. Explain central role
7. α-ketoglutarate might serve as signal of starvation
   1. Li et al (2003) test of α-ketoglutarate as signal for N-starvation
   2. Li et al (2003) experiment
   3. Li et al (2003) result
   4. Li et al (2003): high level of α-ketoglutarate fools *Nostoc*
8. Central question
   1. Maybe plants manipulate α-ketoglutarate in *Nostoc* to simulate starvation?
   2. Does the level of α-ketoglutarate change when *Nostoc* is grown without a source of nitrogen?
9. Experiment
10. Overview of experiment
    1. Measure α-ketoglutarate in *Nostoc* with biosensor
    2. If α-ketoglutarate is a signal, expect higher level in *Nostoc* in plant
11. How to measure α-ketoglutarate? Biosensors
    1. Principle of FRET biosensors (**Figure: FRET cartoon**)
    2. Increase of distance between components decreases fluorescence
    3. Presence of metabolite alters distance
12. Example of FRET use (Hires et al, 2008)
    1. Scientific purpose: detect glutamate near neuron surface
    2. Construction of glutamate-specific FRET
    3. Test of FRET with glutamate (**Figure: fluorescence +/- glutamate**)
    4. Ratio of yellow:blue emission as a measure of glutamate
    5. Actual result: release of glutamate neurotransmitter alters ratio (**Figure: time course of emission ratio change**)
13. Metabolite-specific biosensor for proposal
    1. α-ketoglutarate-specific biosensor doesn’t exist
    2. Glutamate-specific biosensor
    3. Glutamine-specific biosensor instead.
14. Introduction of biosensors into *Nostoc* and *Nostoc* into plant
    1. Cloning of biosensor into plasmid
    2. Introduction of plasmid into *Nostoc* by conjugation
    3. Growth of modified *Nostoc* in plant (*Anthoceros*)
    4. Measurement of glutamate and glutamine
15. Discussion
16. Best possible results
    1. Need for fluorescence ratios to fall within useful ranges
    2. Even so, model predicts α-ketoglutarate levels, not glutamate and glutamine
17. Possibility of α-ketoglutarate biosensor
    1. Three α-ketoglutarate-binding proteins known (α-ketoglutarate dehydrogenase, PII protein, NtcA)
    2. Using binding domains from PII or NtcA may perturb regulation
    3. α-ketoglutarate biosensor outside scope of proposal
18. Problems interpreting results
    1. Cyanobacteria have endogenous fluorescence
    2. Biosensors may not sense biologically relevant levels
    3. Glutamate biosensor may be fooled by aspartate
    4. Calibration difficulties owing to unknown ionic strength in cell
19. Inspirational final words