SAND2017-8187C

Assimilation of lignin-breakdown products by lignolytic and non-lignolytic bacteria and fungi

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U.S. DEPARTMENT OF ENERGY
NNSA National Nuclear Security Administration

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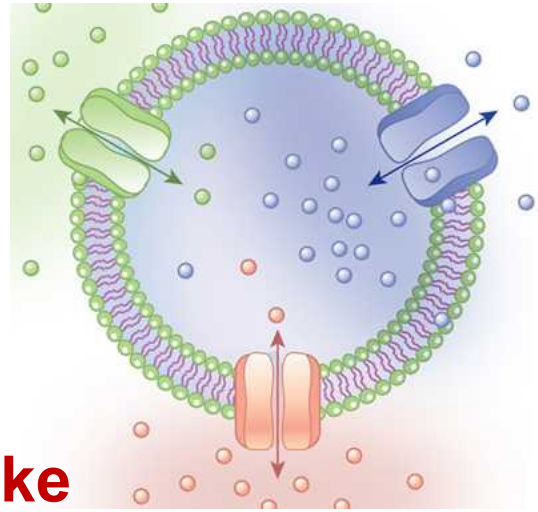
Motivation

- *Engineered system capable of tailored transport of lignin fragments*
- *Transport of lignin fragments across cell membrane is virtually unknown*
- *Important step with potential to limit efficiency*
- *Needs:* *substrate range*
  substrate specificities
  kinetics



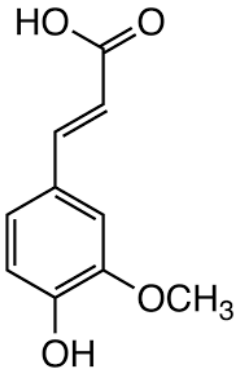
Comprehensive study of native uptake

Experimental Design

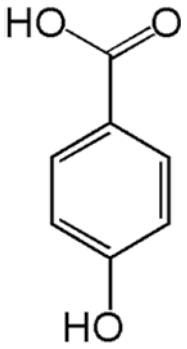
- 6 lignin fragments
- 4 organisms
- 3 replicates
- Mass spectrometry
- HPLC
- Single cell microscopy

Lignin Fragments

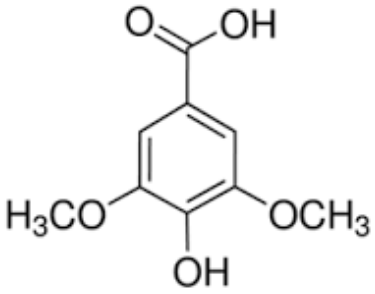
ferulic acid



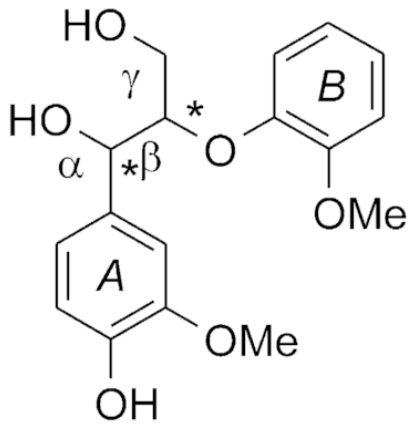
4-hydroxybenzoic acid



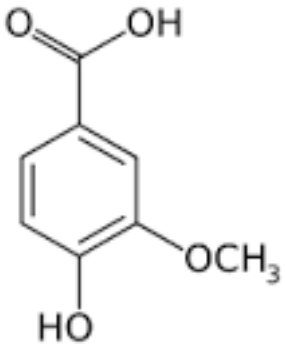
syringic acid



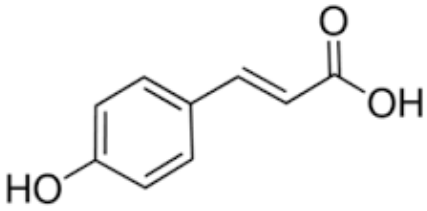
guaiagylglycerol beta-guaiacyl ether (GGE)



vanillic acid



p-coumaric acid



Microbes

	Non-lignolytic	Lignolytic
Bacteria	*E. coli*	*E. lignolyticus SCF-1*
Fungi	*S. cerevisiae*	*P. chrysosporium*

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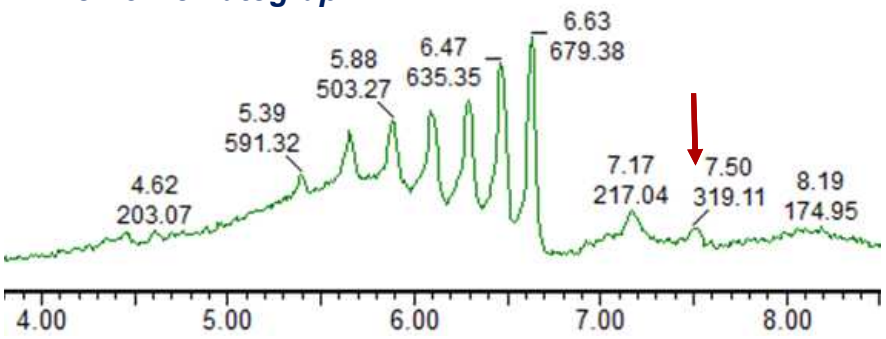
Determines intracellular concentration of lignin breakdown products

LC-MS

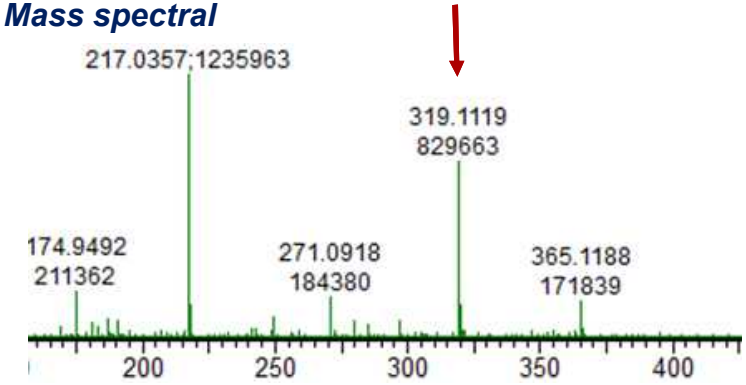
LC-MS Method

- Column: F5 (pentafluorophenyl) reverse phase
- Gradient: 0.1% formic acid + water to 0.1% formic acid + 95% acetonitrile, 20 min
- MS: negative mode

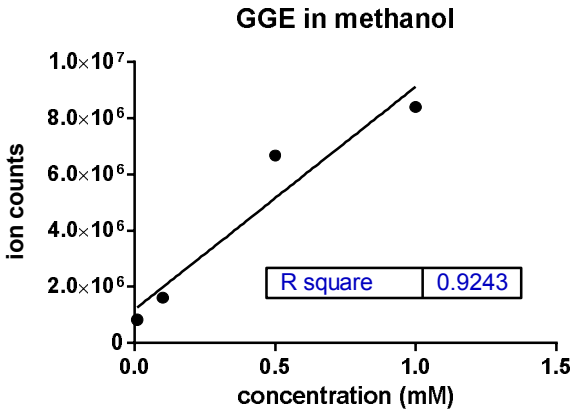
Ion chromatograph

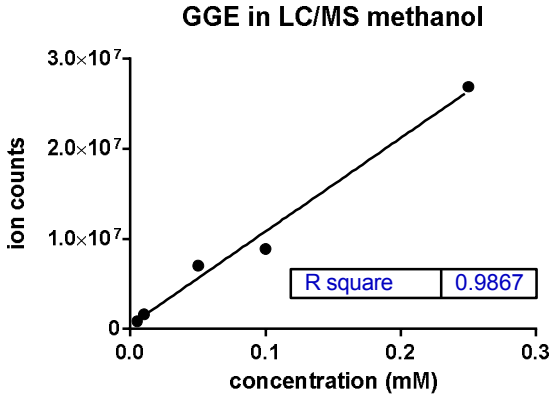


Mass spectral



GGE uptake in yeast





LC-MS: Direct Evidence of Transport

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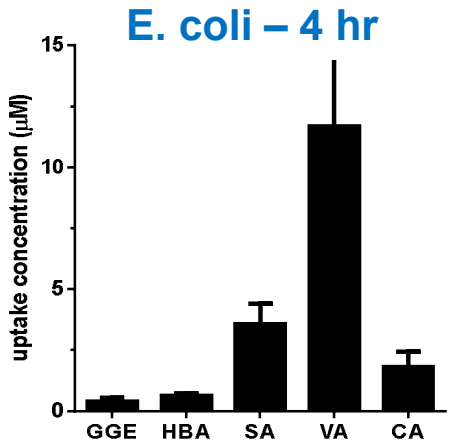
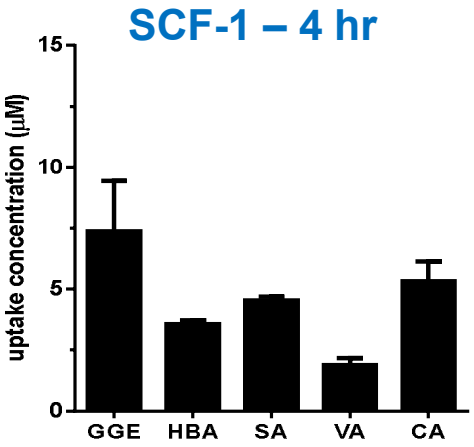
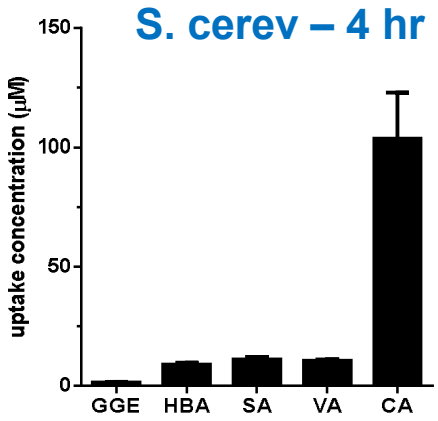
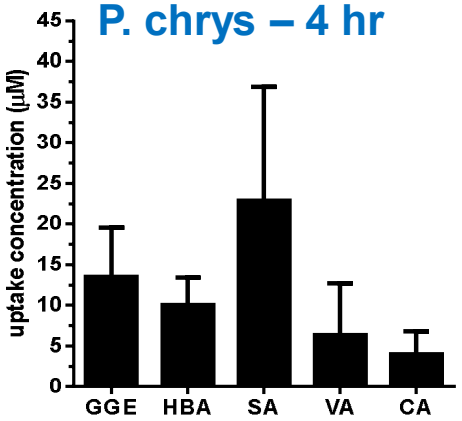
Non-lignolytic vs. Lignolytic

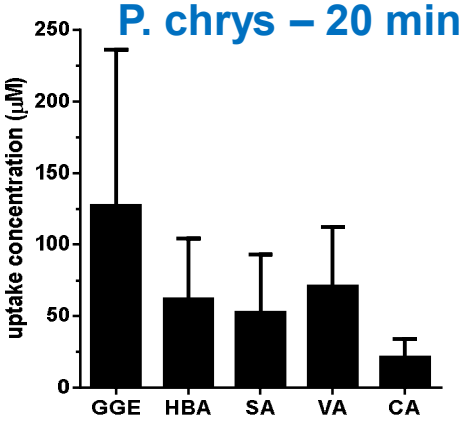
Fungal vs. Bacterial











- **A diaryl goes in (and a lot of it)**
- **Yeast love coumaric acid**
- **20 min concentrations > 4 hrs -> Metabolism**

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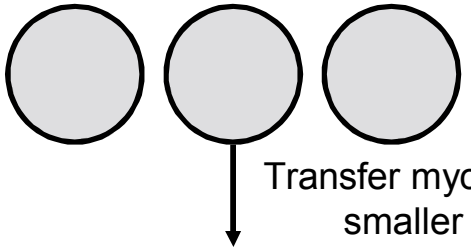
Determines concentration remaining in media

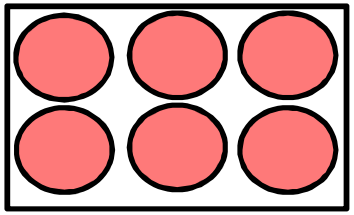
HPLC

HPLC Method

P. chrysosporium grown in lignolytic-inducing conditions for 5 days in fungal media

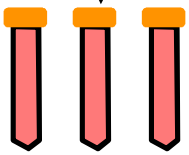


Transfer mycellium to smaller well



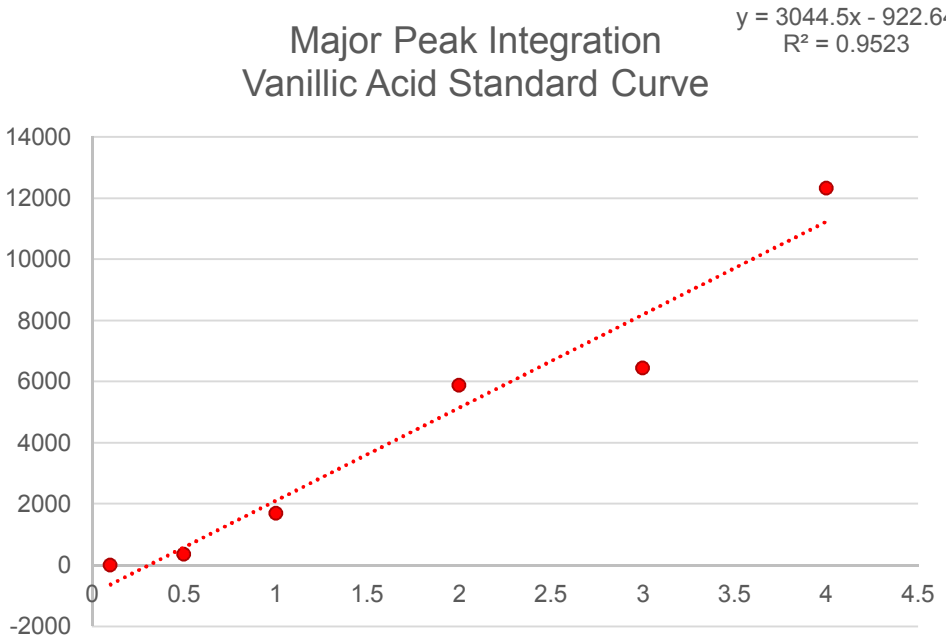
+ Mono/di-lignols in PBS

Allow uptake for 72 hours

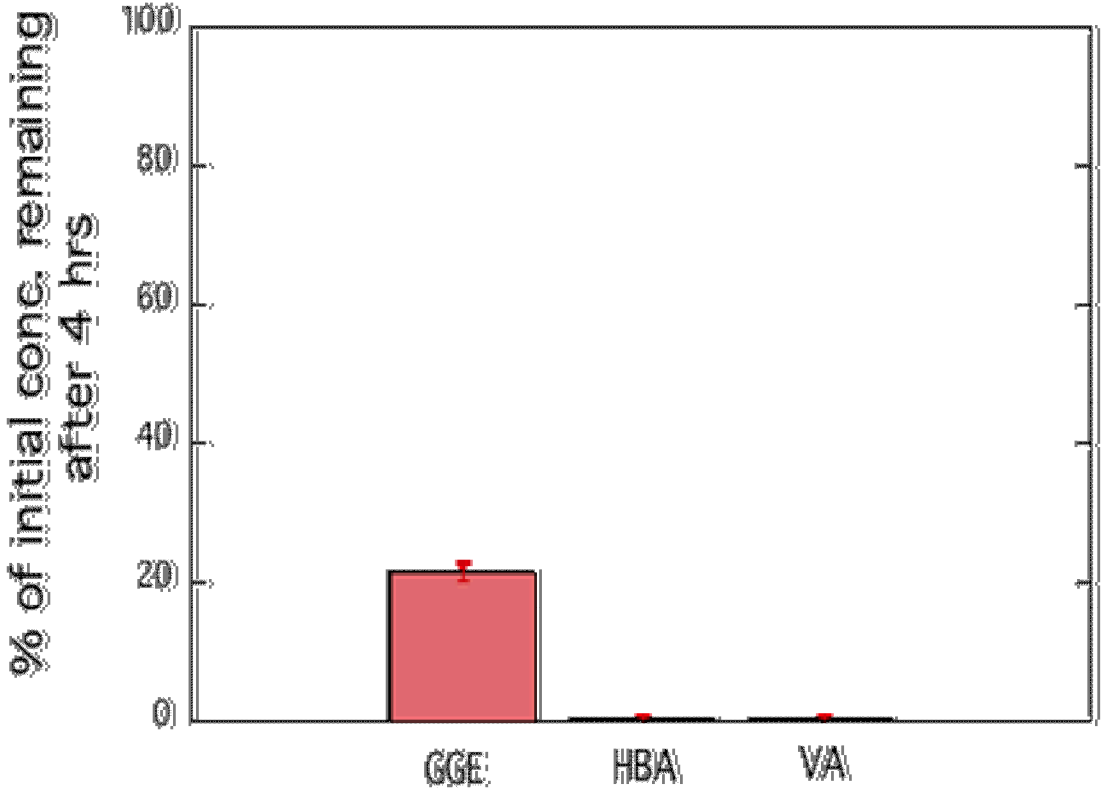


Harvest supernatant and run on HPLC

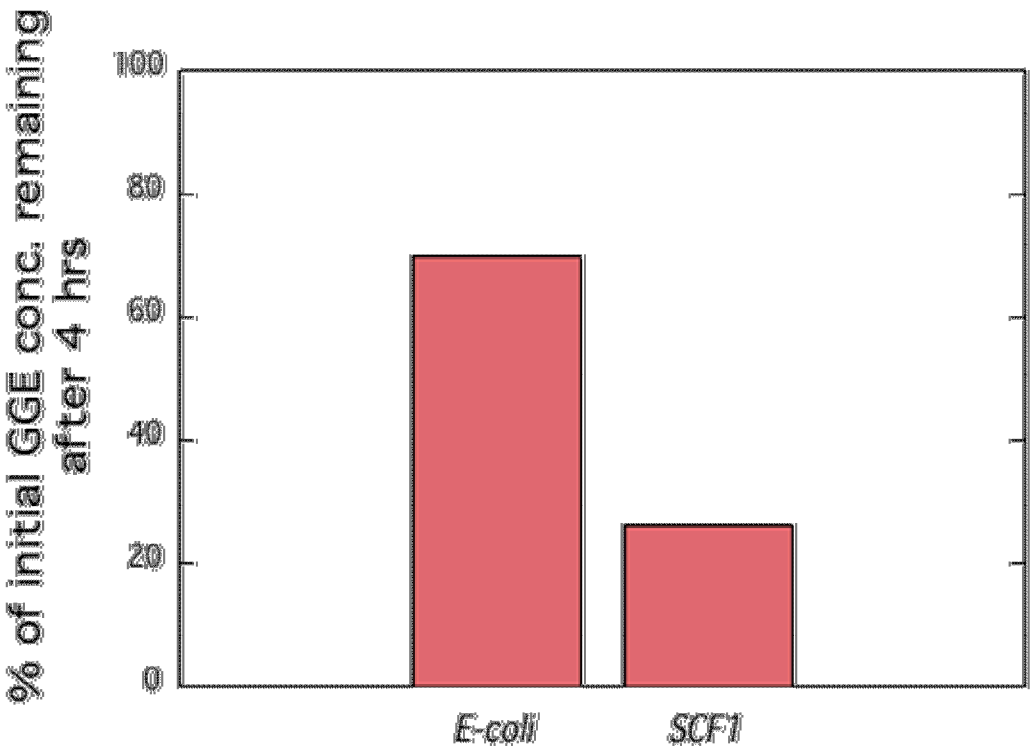
Detect mono/di-lignol concentration remaining after 72 hours uptake



Fungi Transport



Bacteria too!



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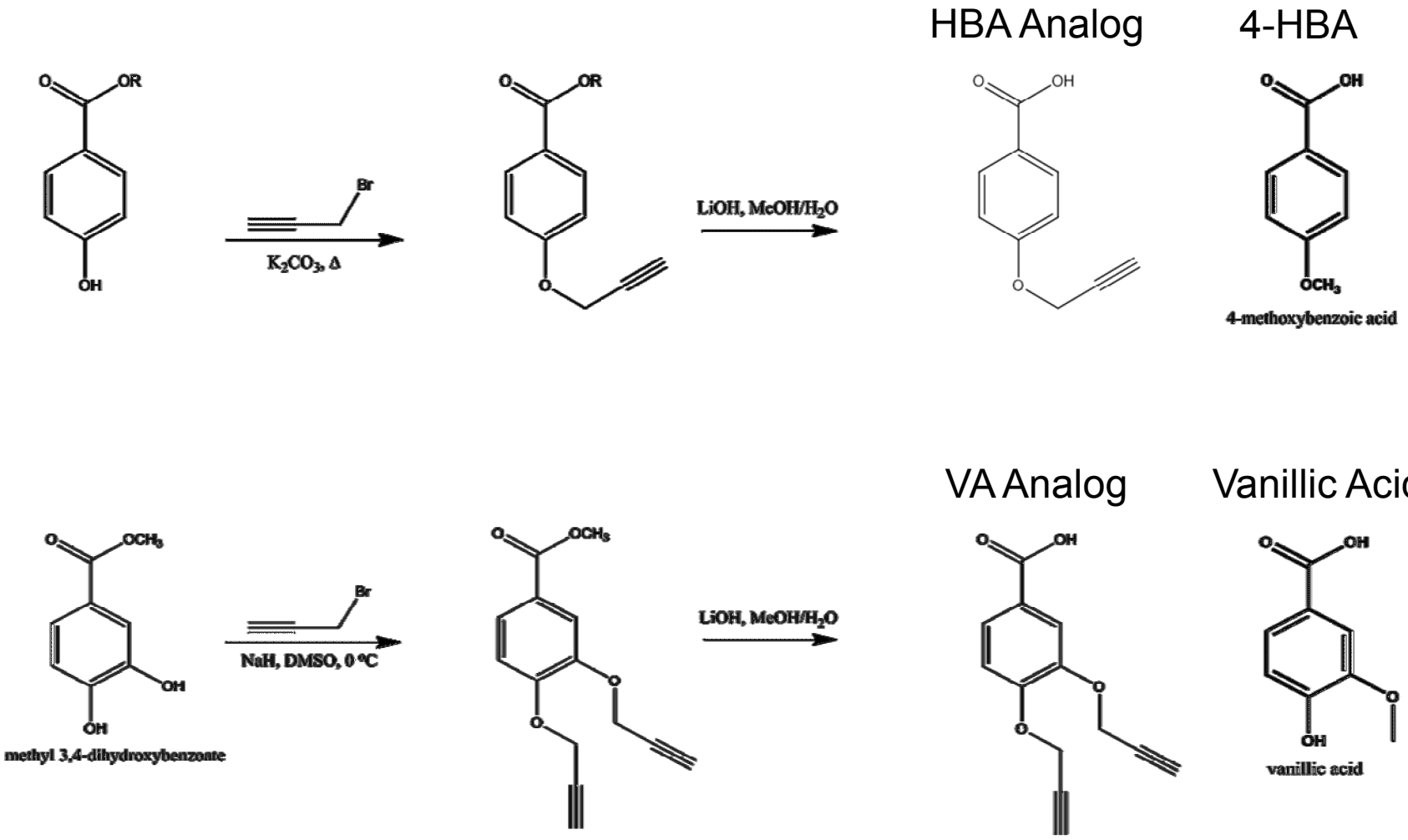
Reveals localization and uptake heterogeneity

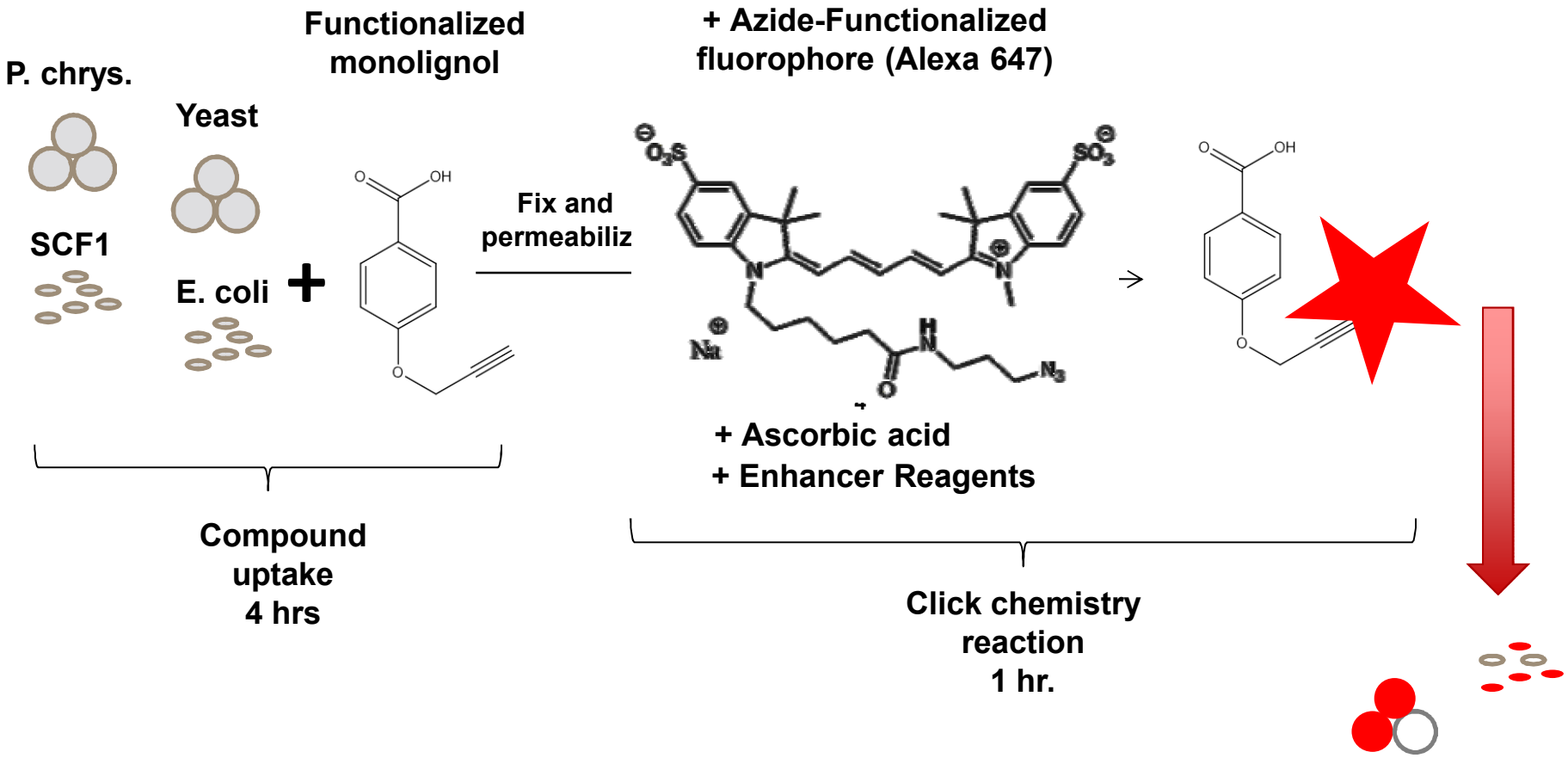
SINGLE CELL ANALYSIS

Alkyne Functionalized Substrates



Tobimatsu Y, Davidson CL, Grabber JH, Ralph J. 2011. *Biomacromolecules* 12: 1752-61

Labeling monolignol alkyne-functionalized substrates



Microscopy Results

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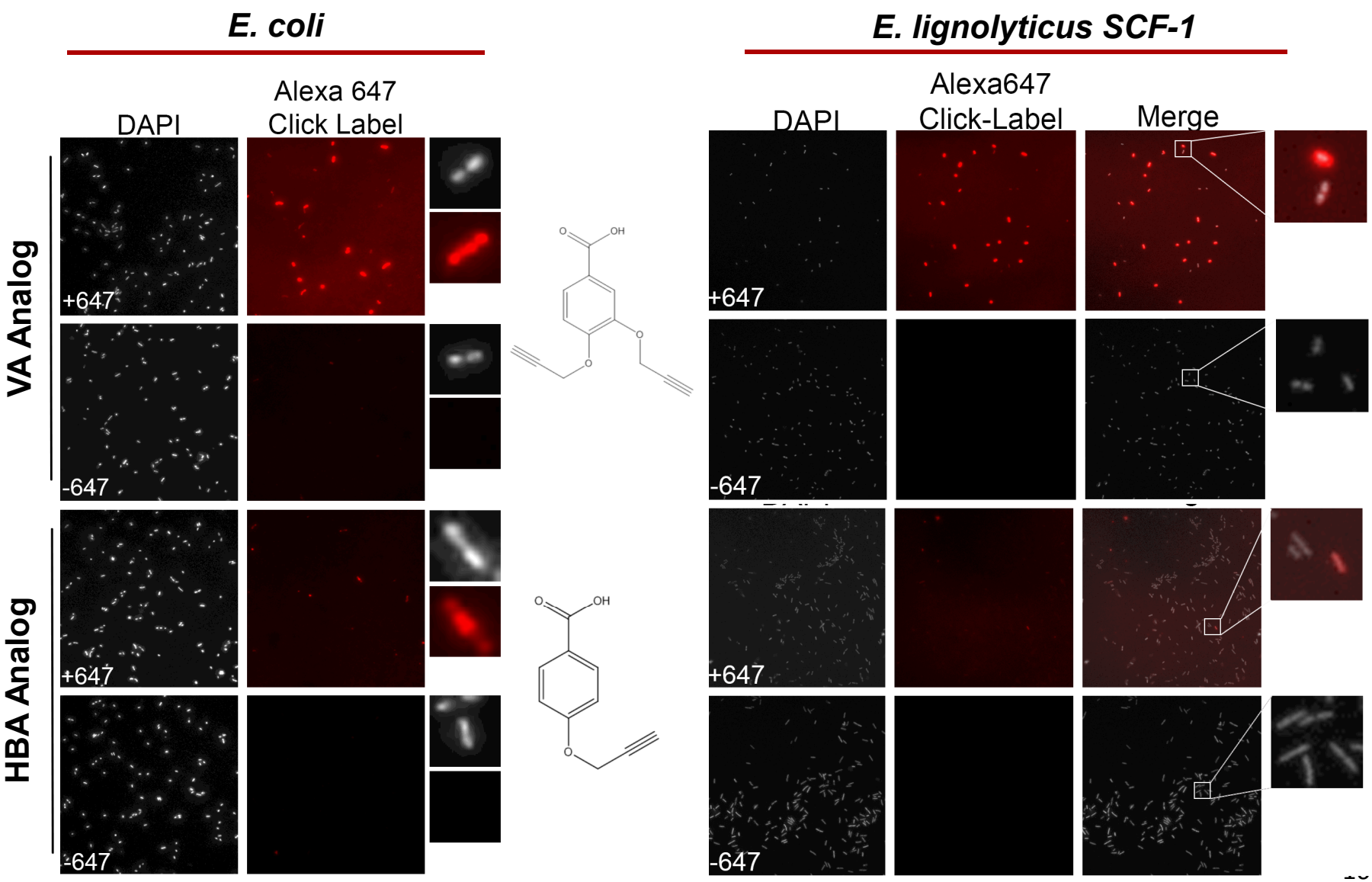
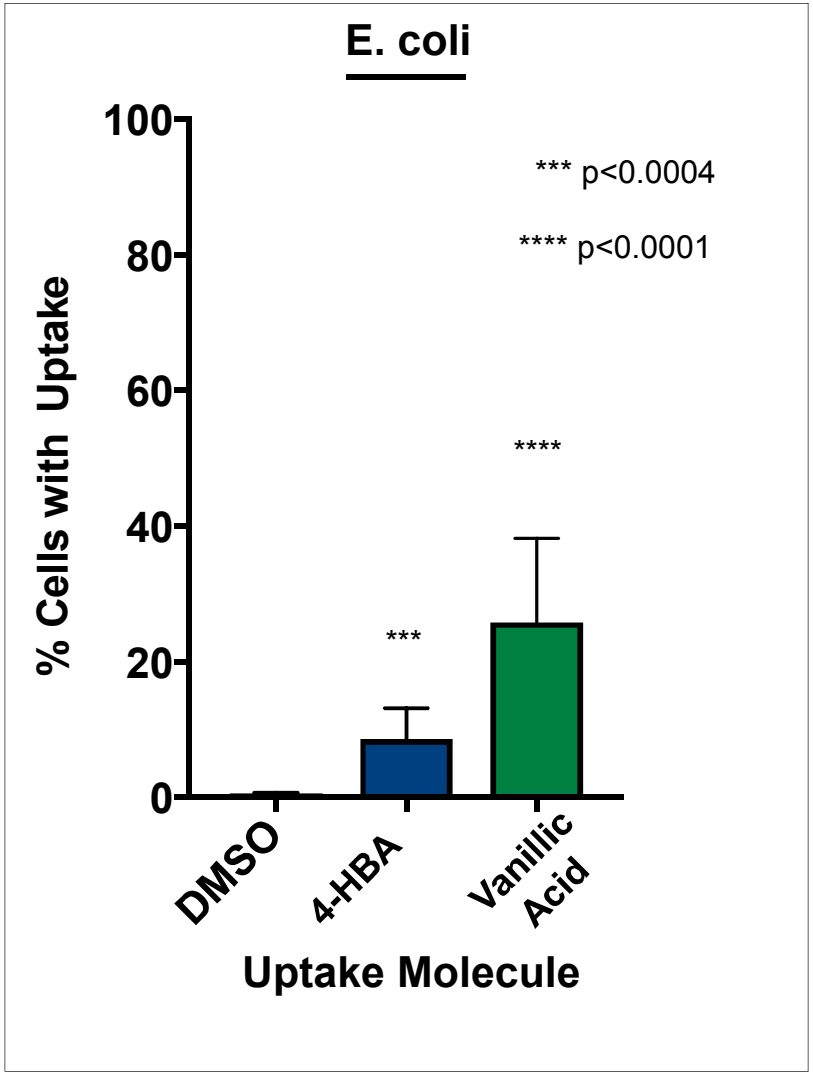
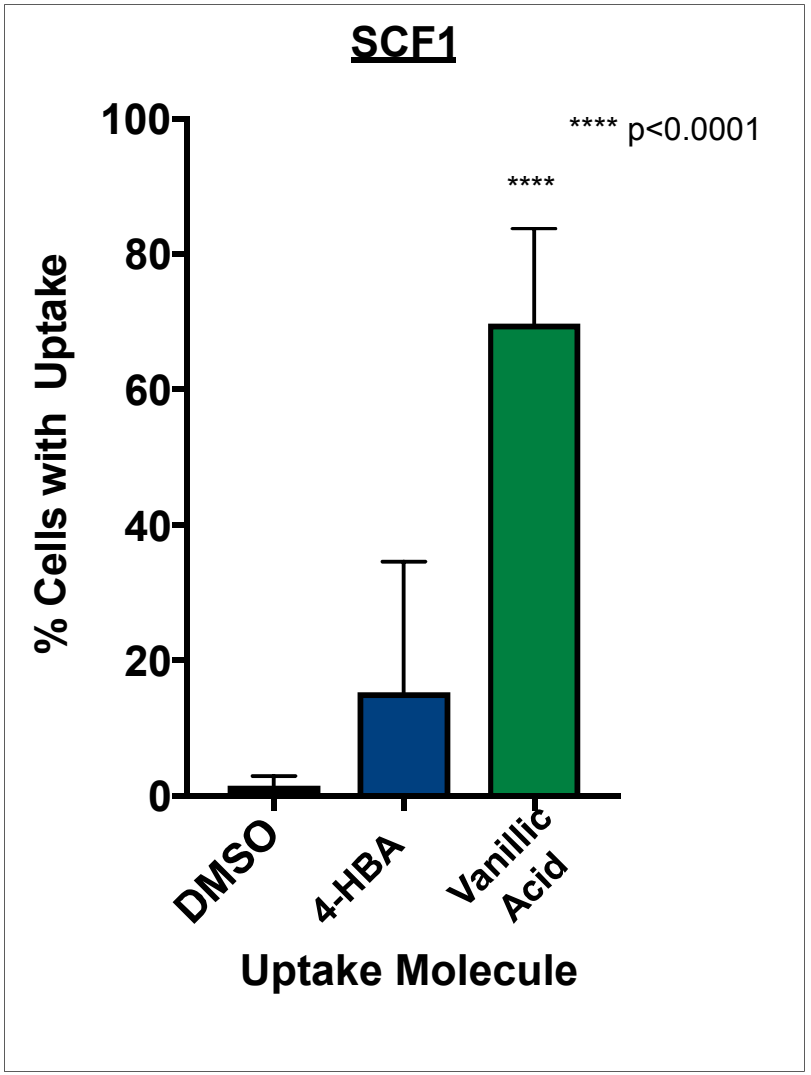


Single Cell Uptake Analysis





SCF-1 Exhibits Aspect Ratio Change

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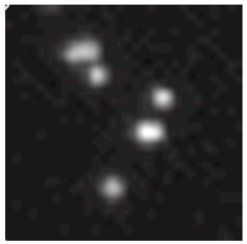
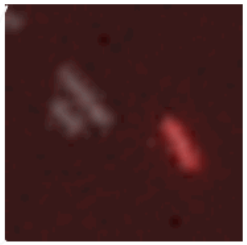
DMSO Control

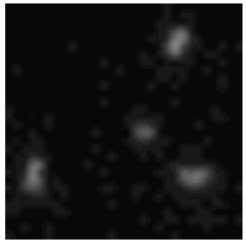
HBA Analog

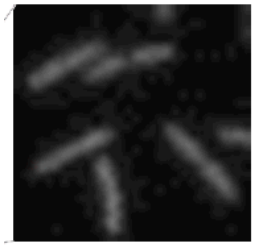
+ A647

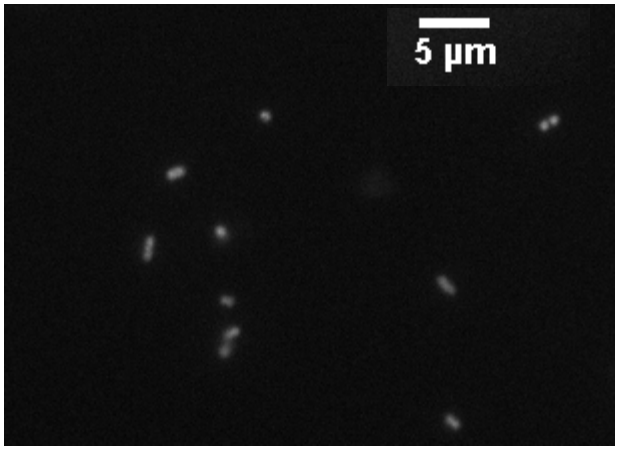


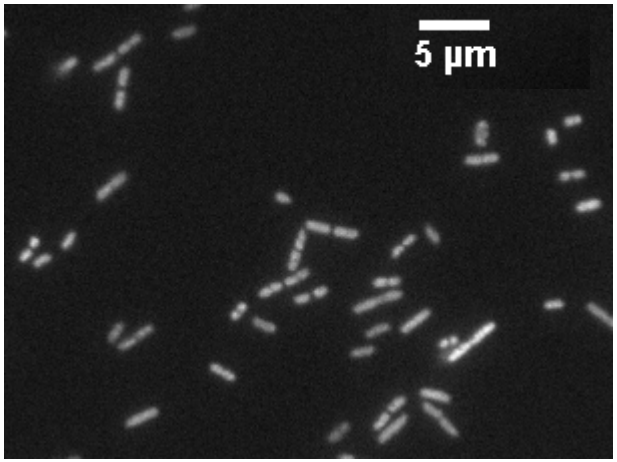


- A647









Microscopy Results

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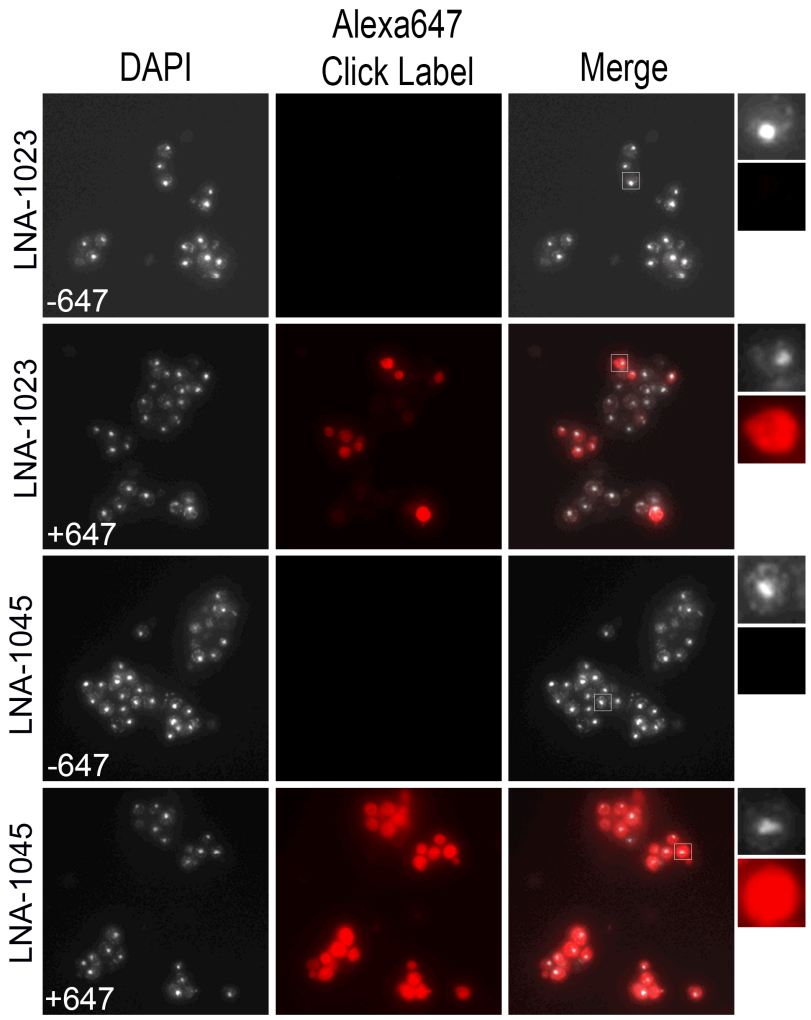
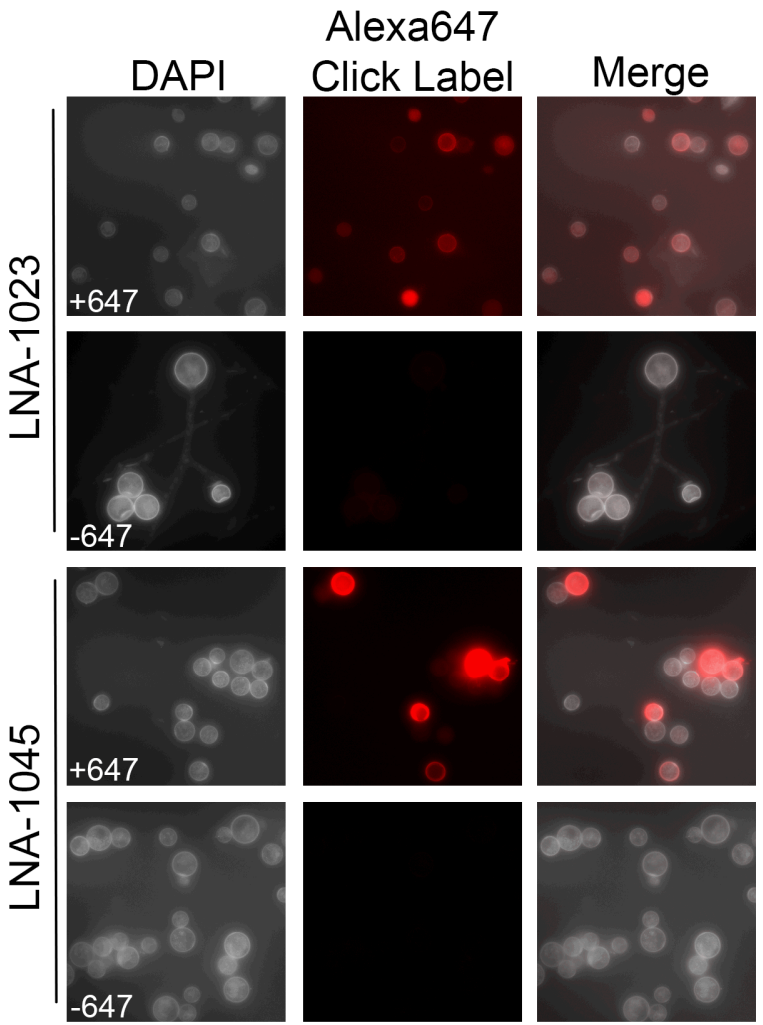
S. cerevisiae



P. chrysosporium



Conclusions

- Direct evidence of uptake and metabolism of lignin fragments in lignolytic and non-lignolytic fungi and bacteria
  - LC-MS
  - Single-cell fluorescence microscopy
- Indirect evidence of metabolism
  - HPLC, LC-MS
- Differential uptake and metabolism observed

Acknowledgments

- Kristen D. Angelis, University of Massachusetts
- John Ralph, University of Wisconsin
- John Gladden, SNL & JBEI

- Danae Maes & Troy Simoes, undergraduate interns

LDRD
Laboratory Directed Research and Development

P. chrysosporium Transport compounds