

Foundational advances in RNA engineering applied to control of biosynthesis

Christina D. Smolke

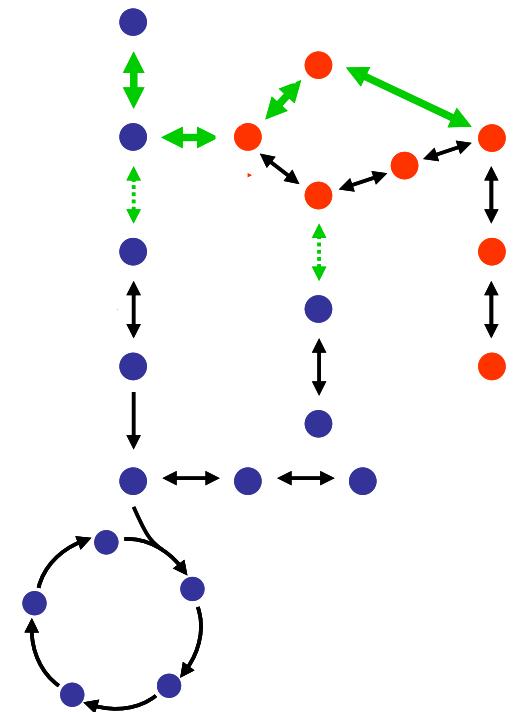
Division of Chemistry and Chemical Engineering
California Institute of Technology

February 11, 2008

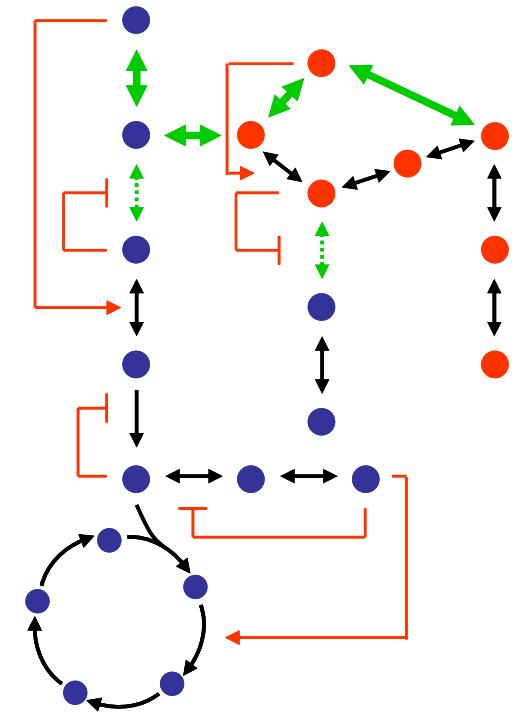
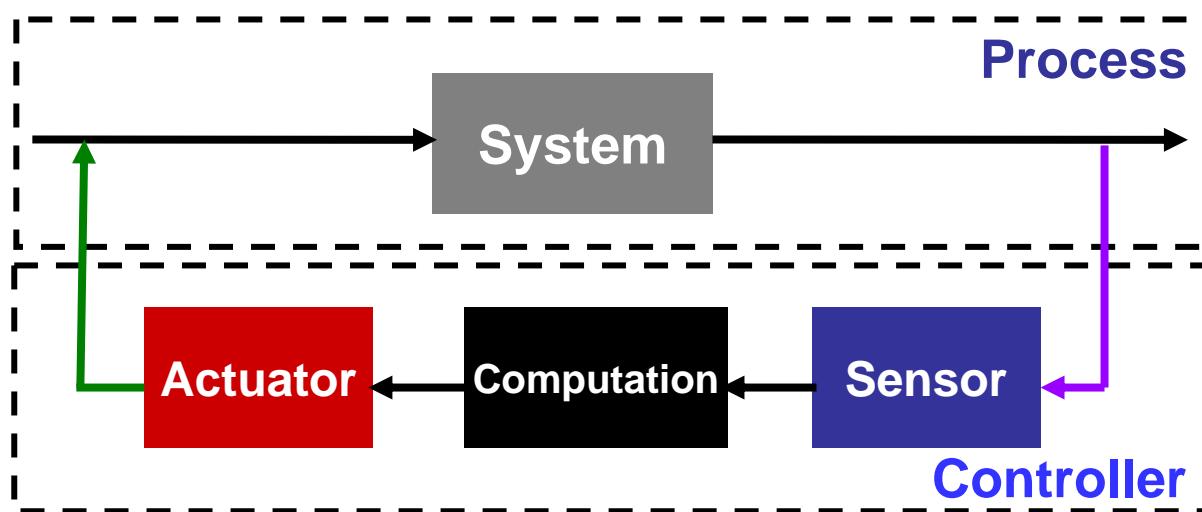
MEWG: Interagency Conference on Metabolic Engineering



The role of feedback control in biosynthesis

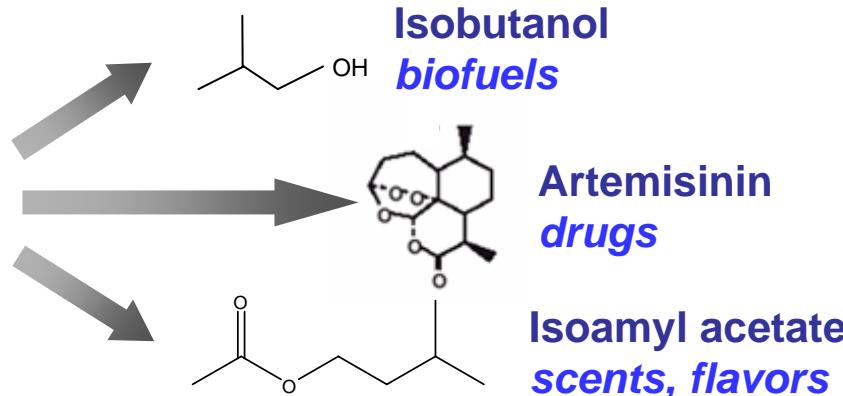
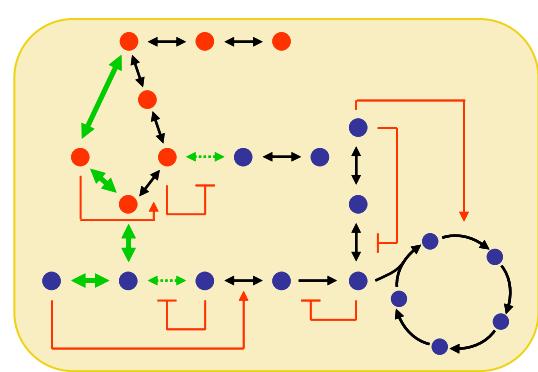


The role of feedback control in biosynthesis

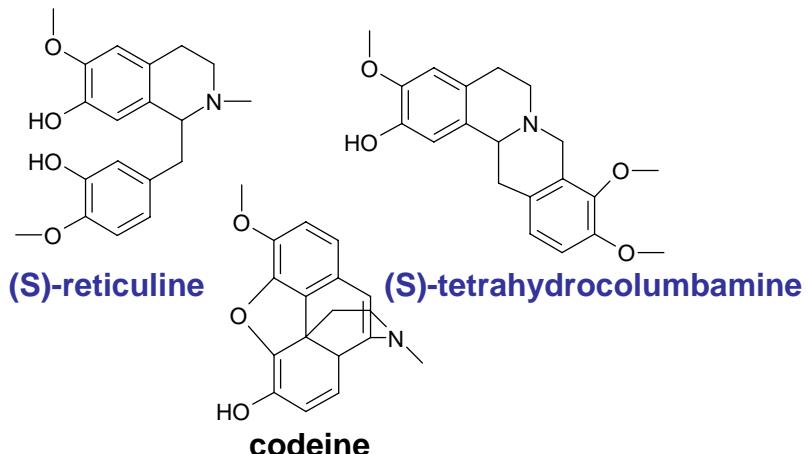


Applications of engineered biological systems

Metabolic engineering



Benzylisoquinoline alkaloid biosynthesis



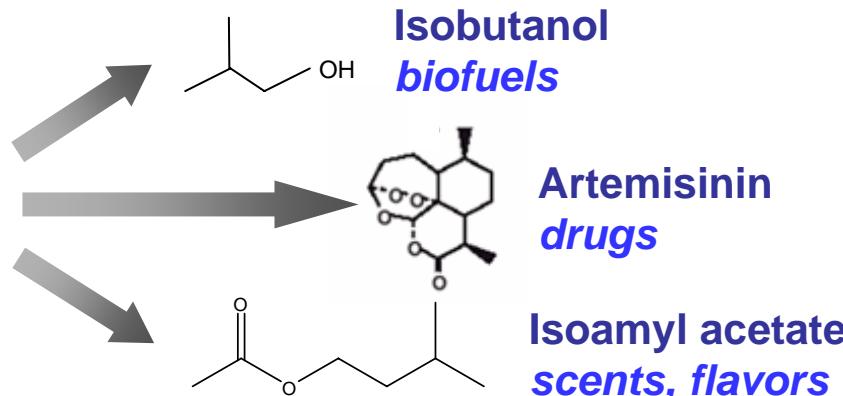
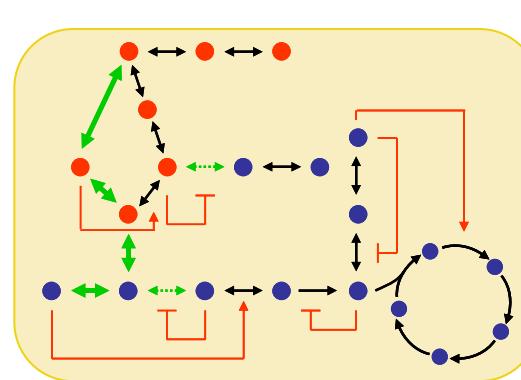
Papaver somniferum (opium poppy)

<http://www.nature.com/news/2004/040816/images/yeast.jpg>

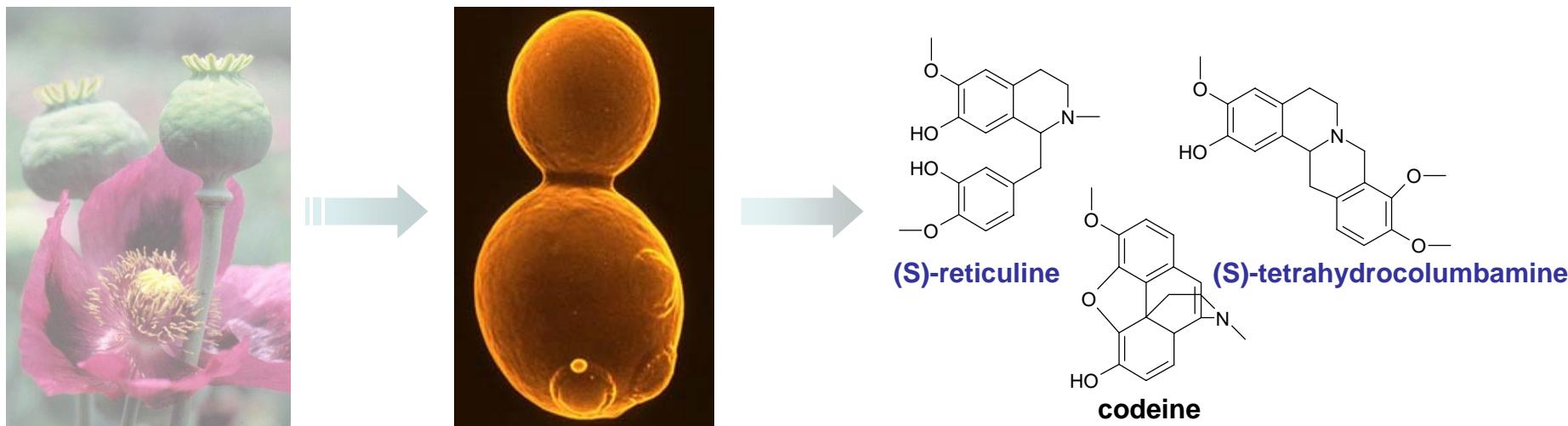
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Applications of engineered biological systems

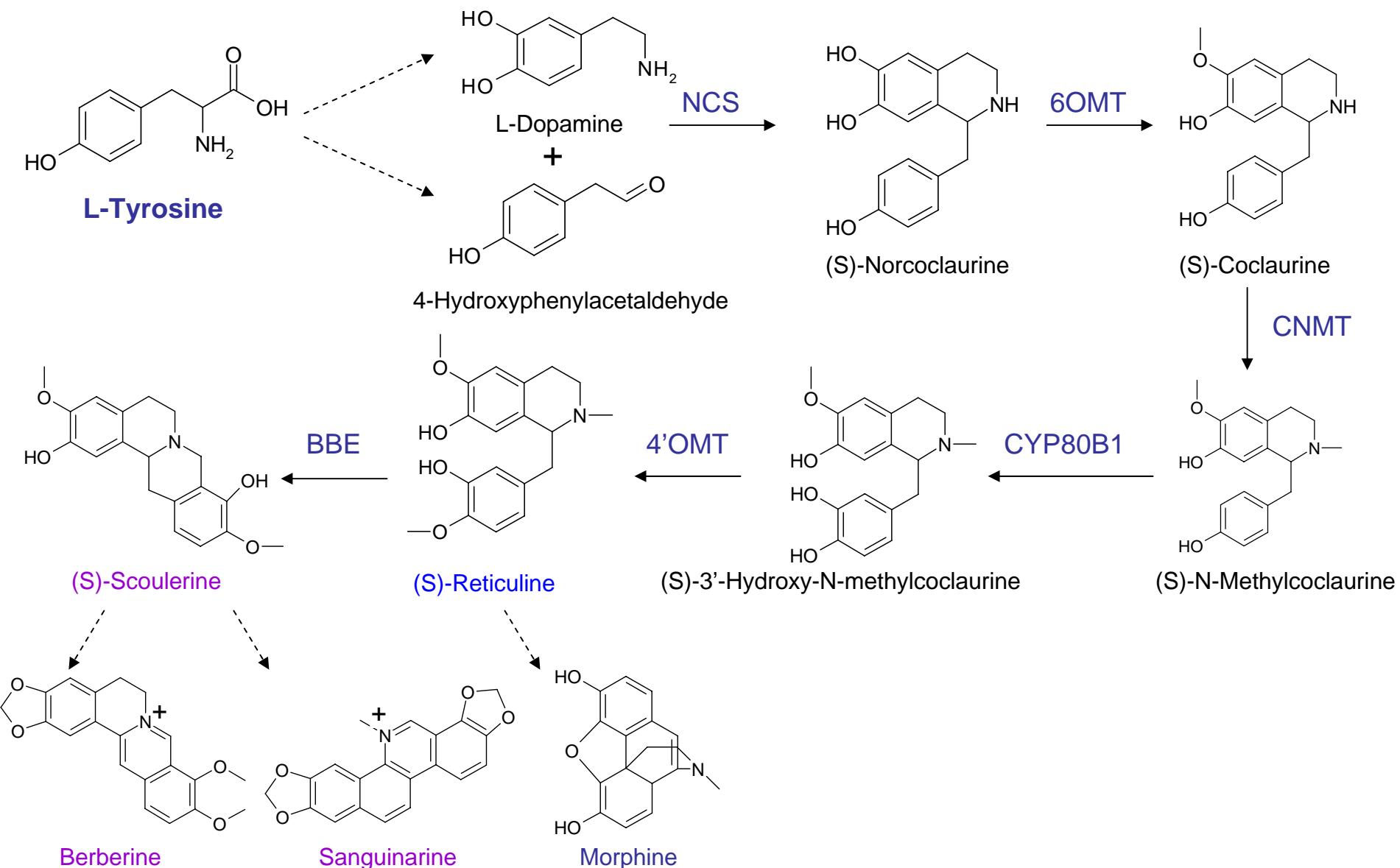
Metabolic engineering



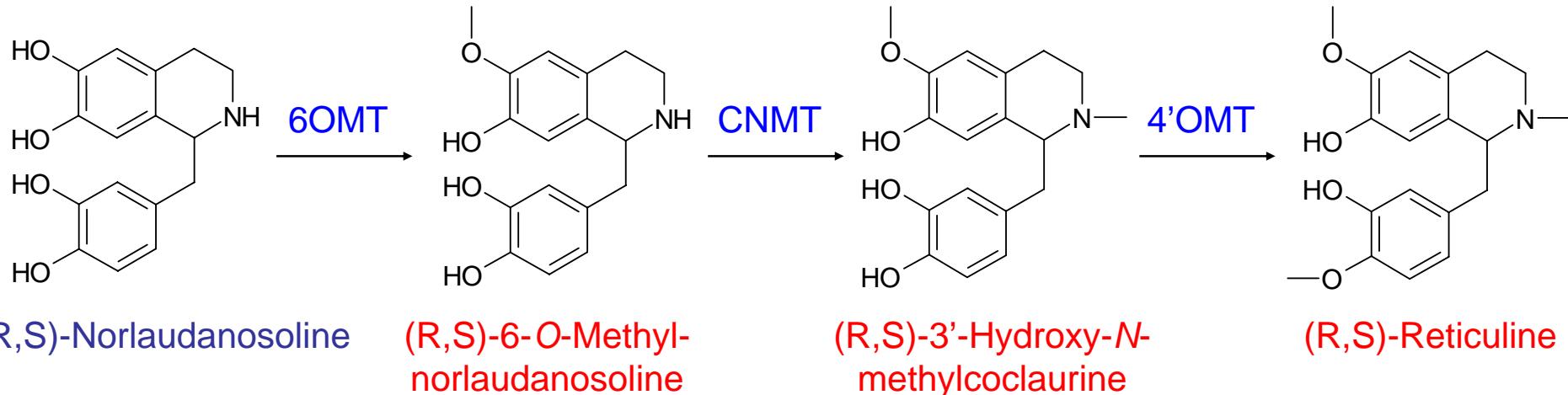
Benzylisoquinoline alkaloid microbial synthesis



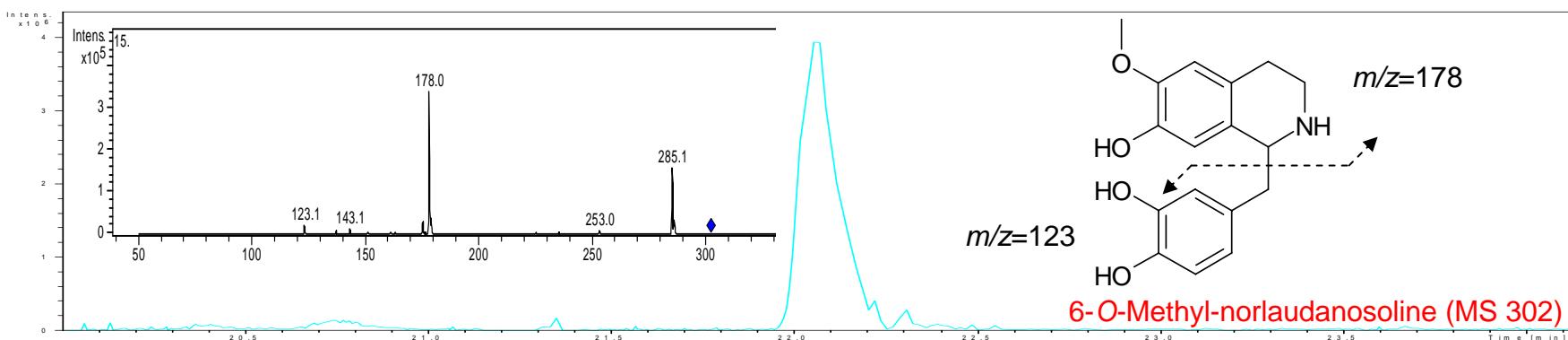
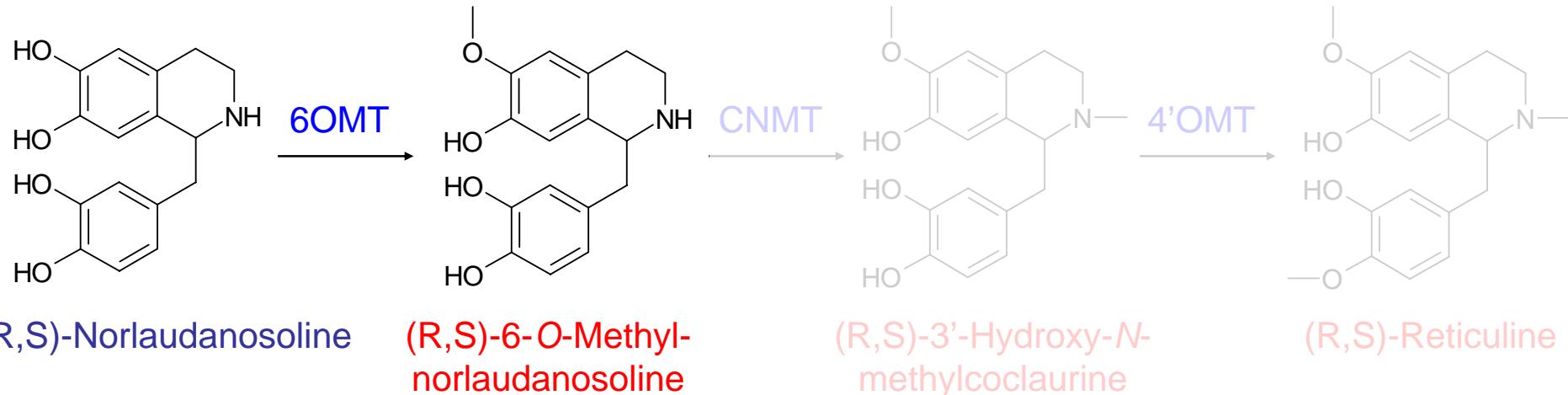
Native BIA pathway



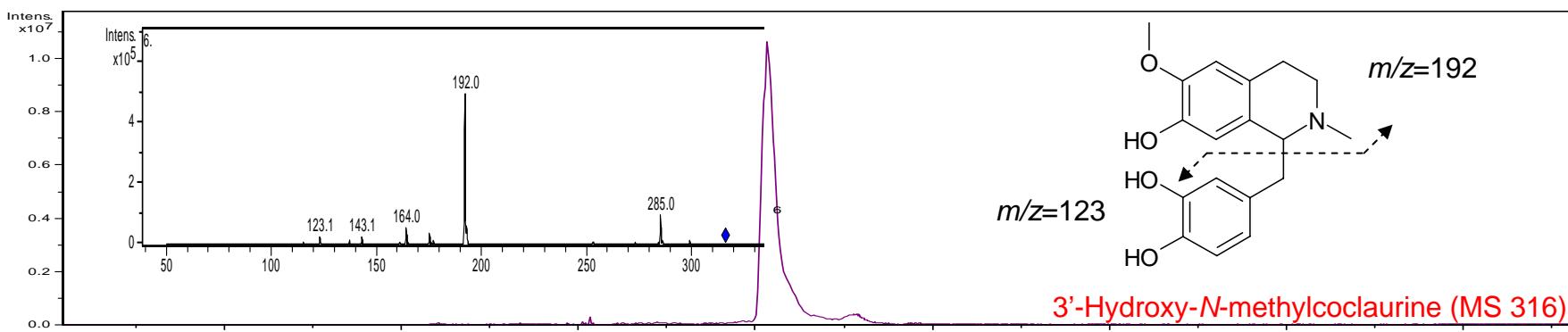
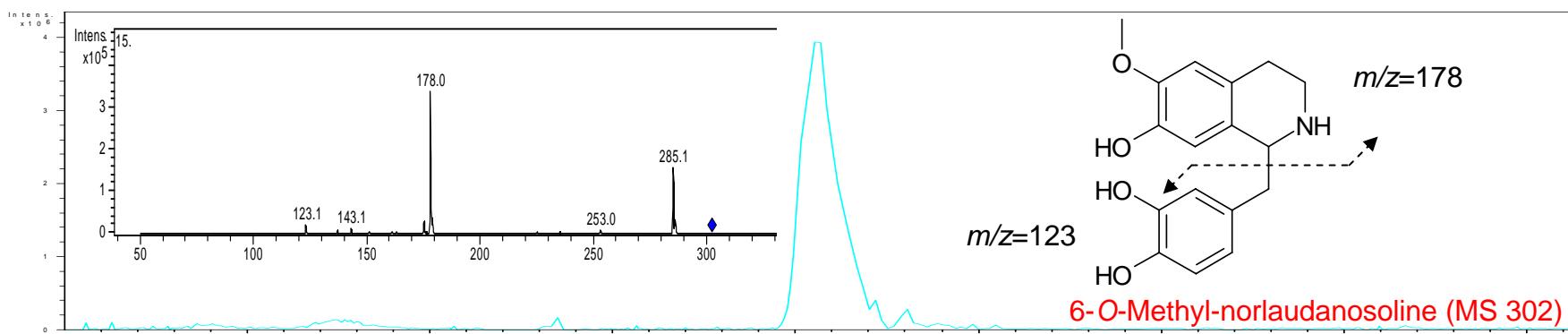
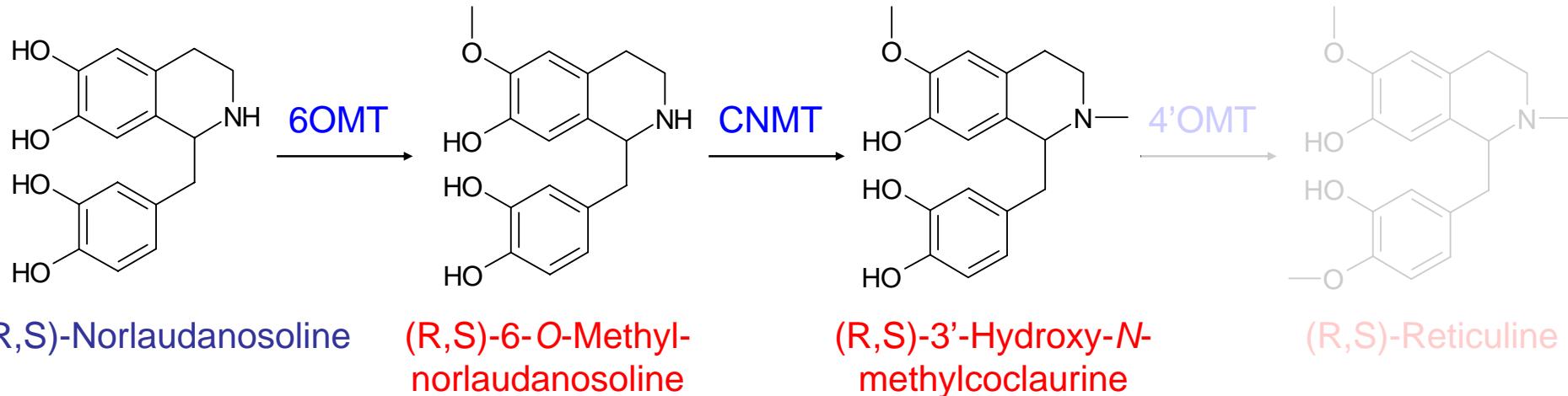
Late Synthetic BIA pathway



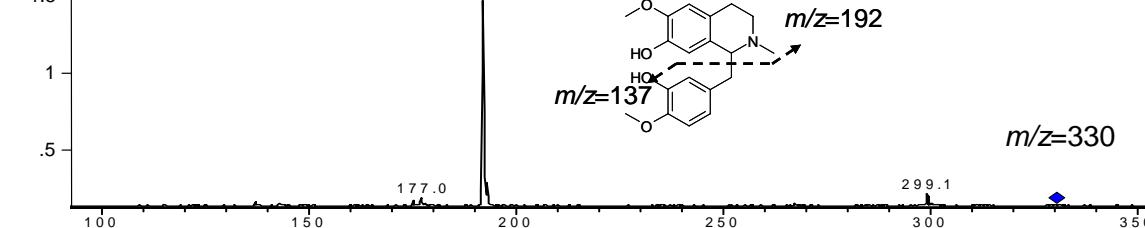
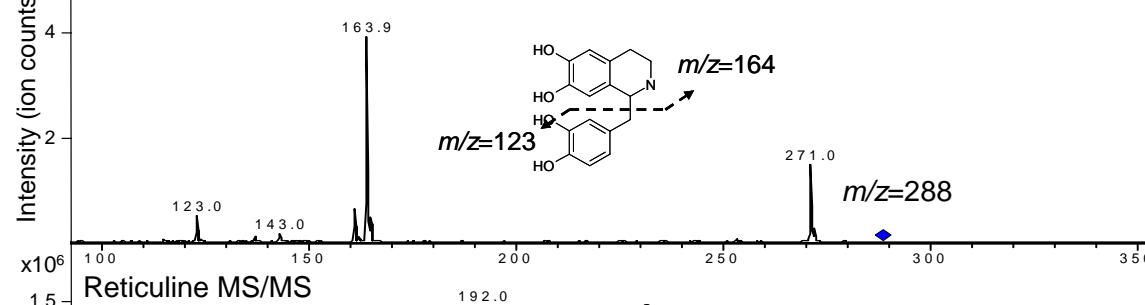
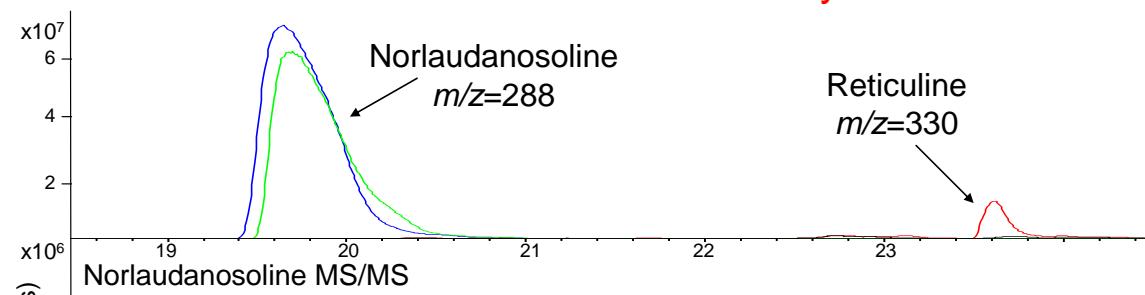
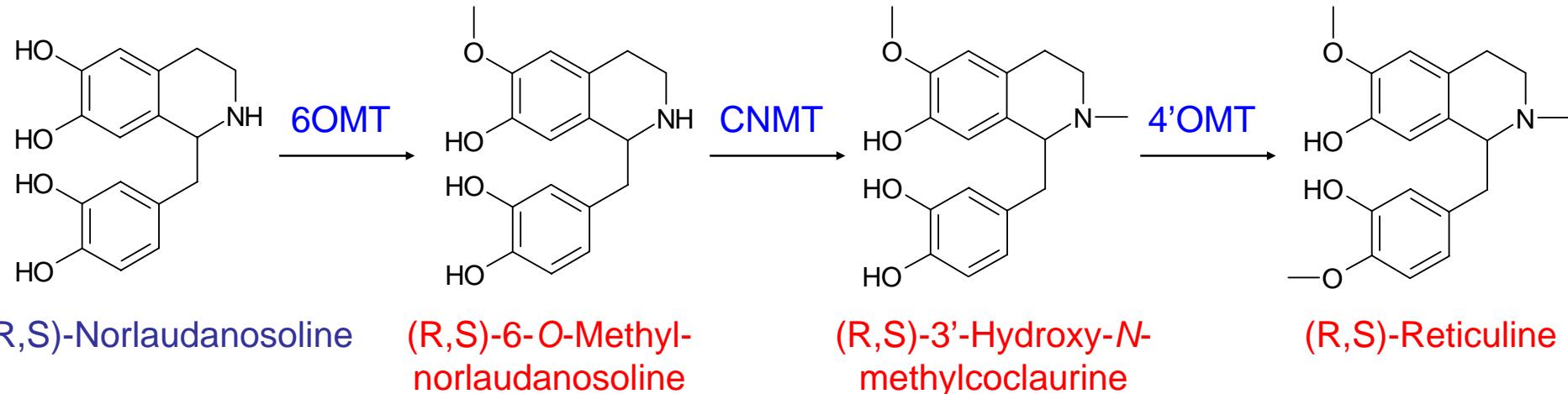
Production of 6-O-Methyl-norlaudanosoline



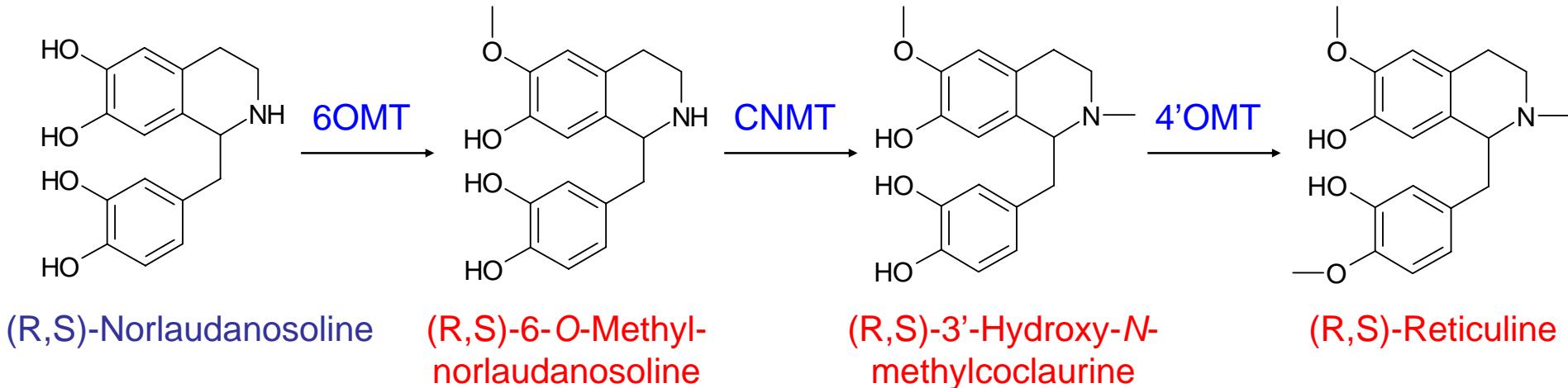
Production of 3'-Hydroxy-N-methylcoclaurine



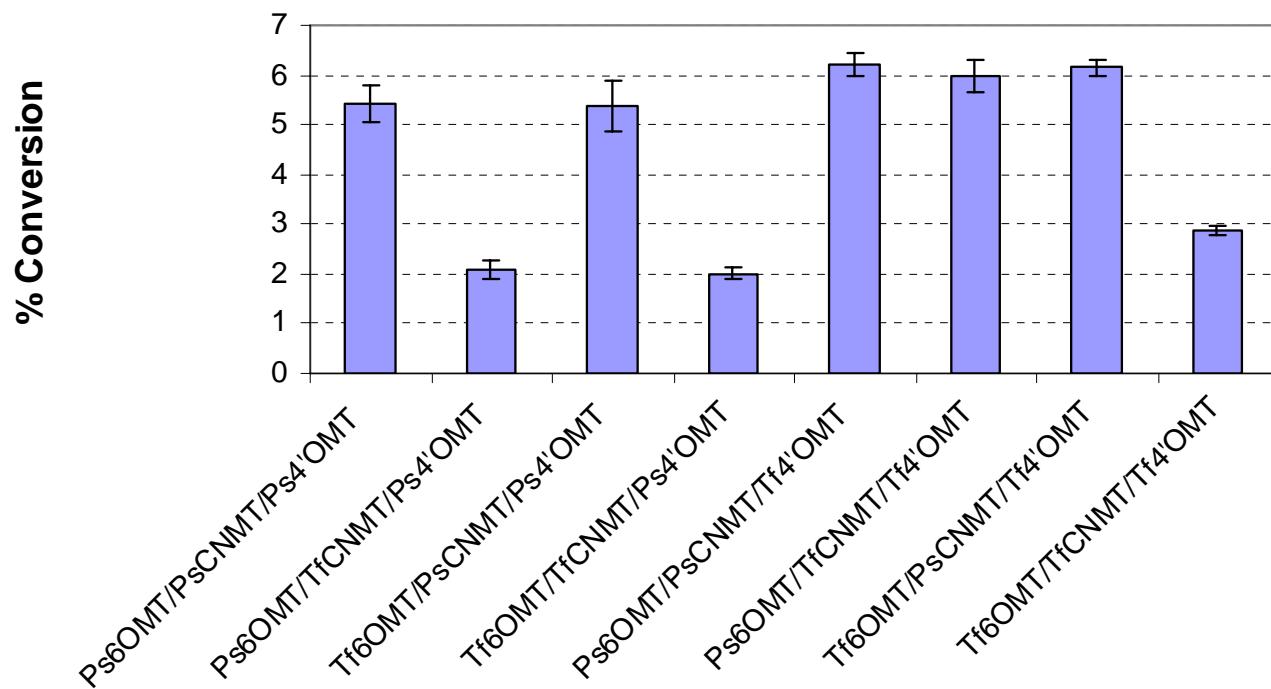
Production of (R,S)-Reticuline



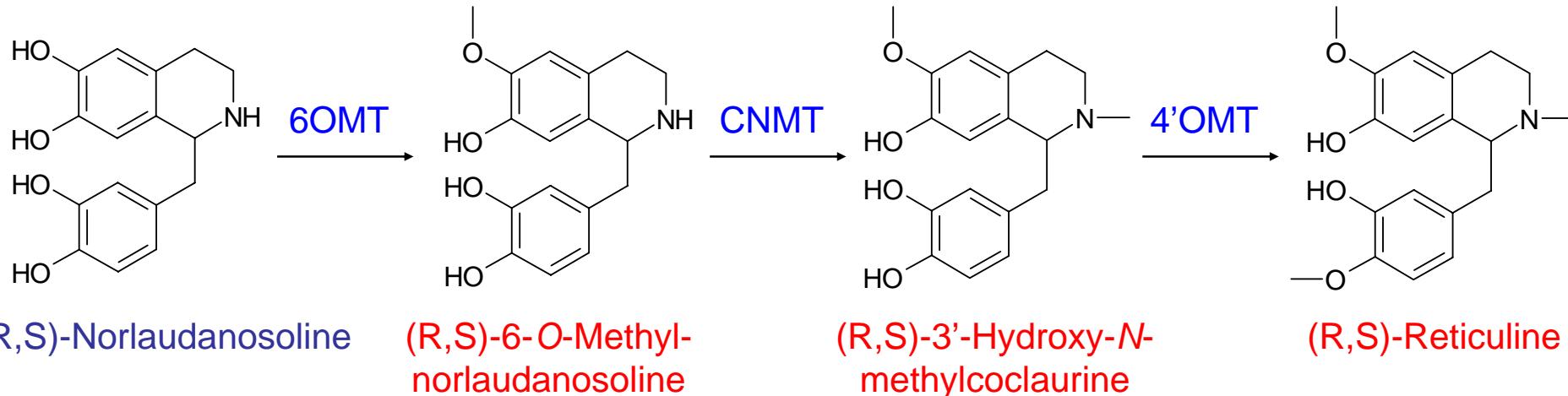
Optimization of (R,S)-Reticuline production



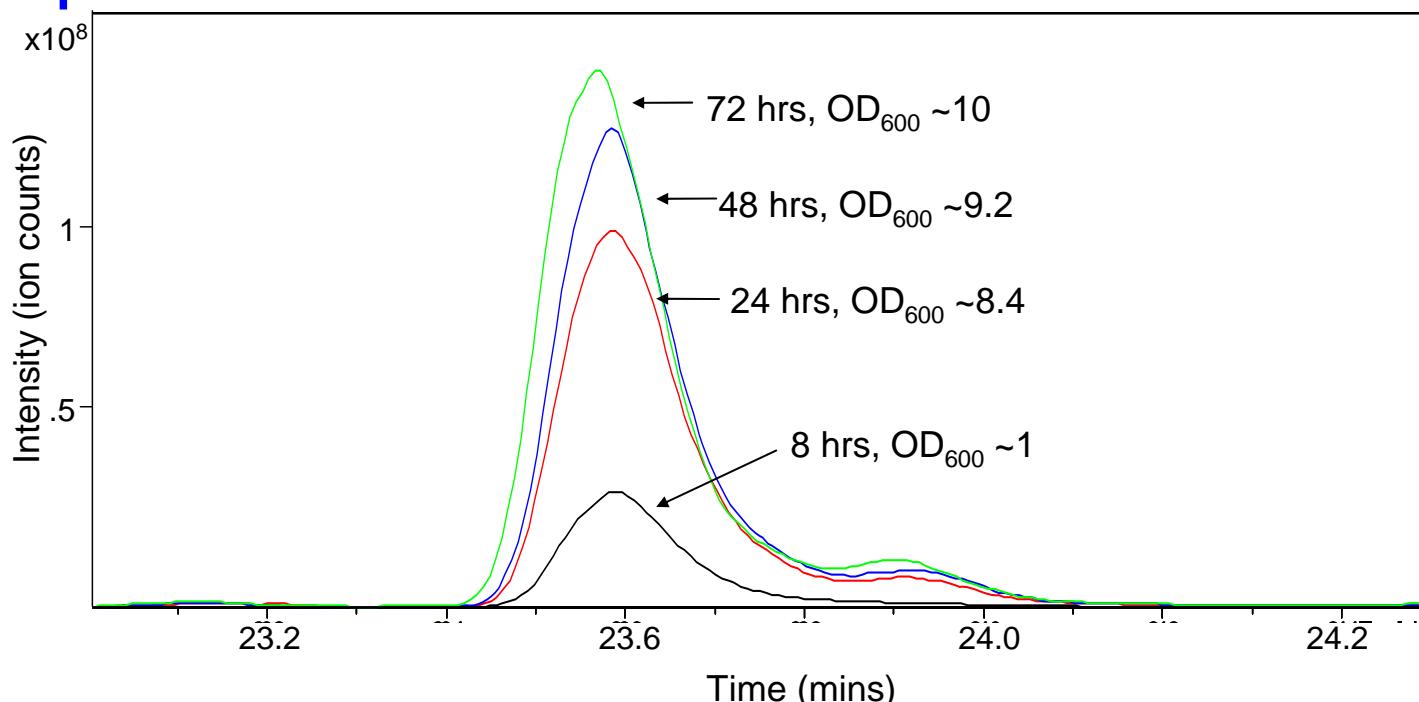
Enzyme variant dependence:



Optimization of (R,S)-Reticuline production

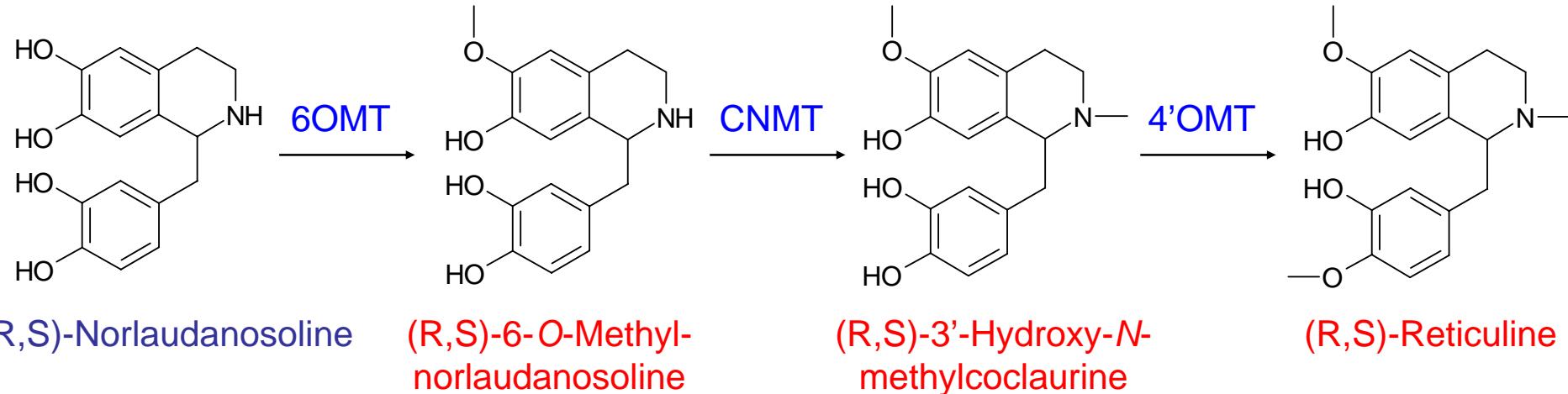


Time dependence:

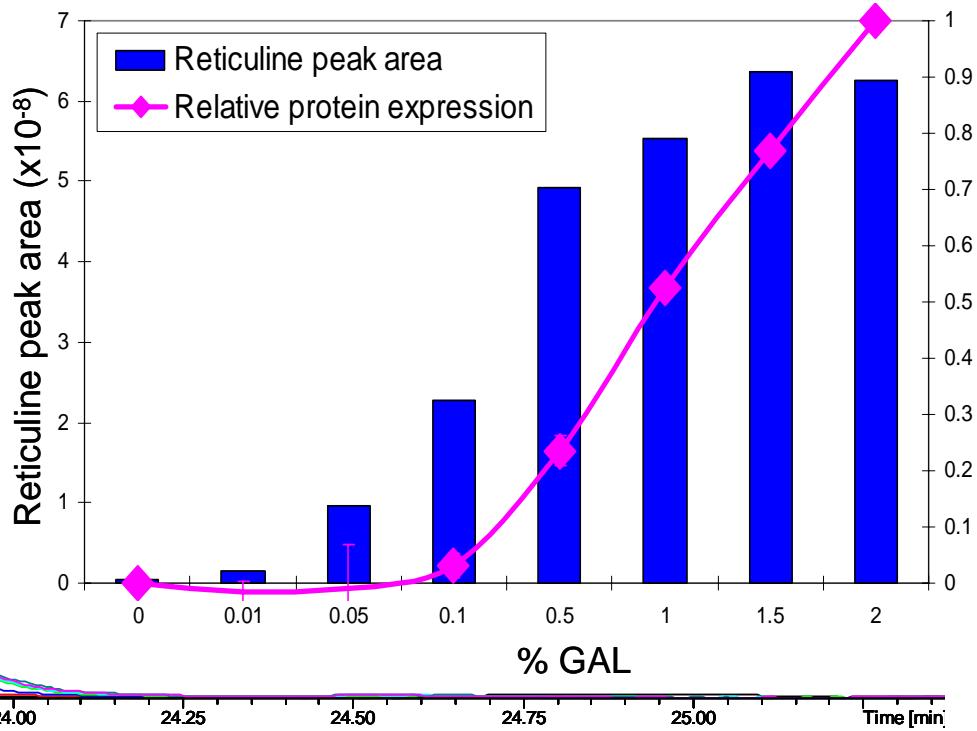
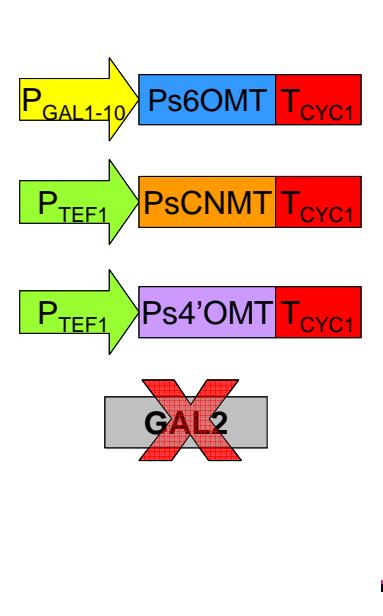


Strains continue to accumulate (R, S)-Reticuline during growth in stationary phase ($OD_{600} \sim 10$).

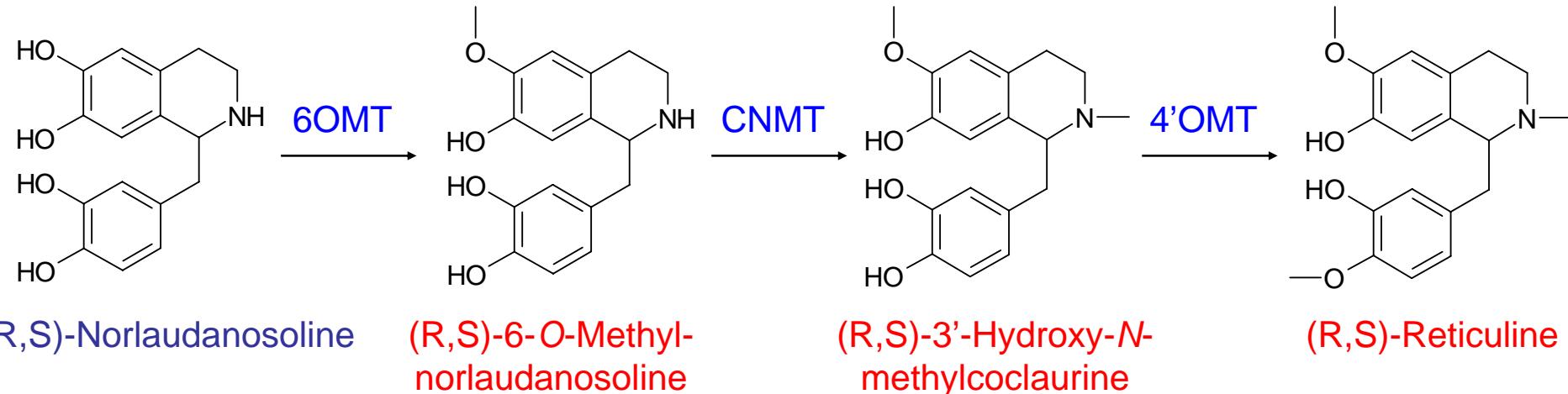
Optimization of (R,S)-Reticuline production



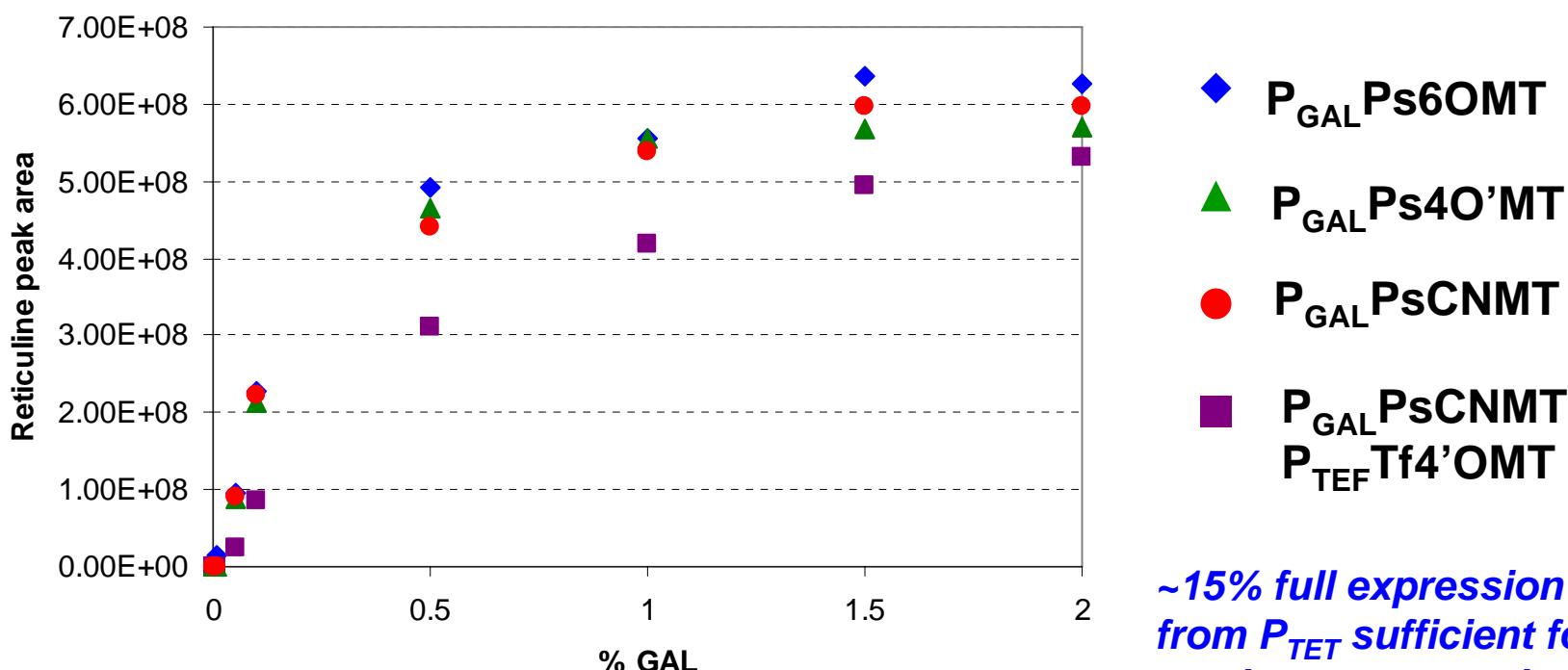
Enzyme level dependence:



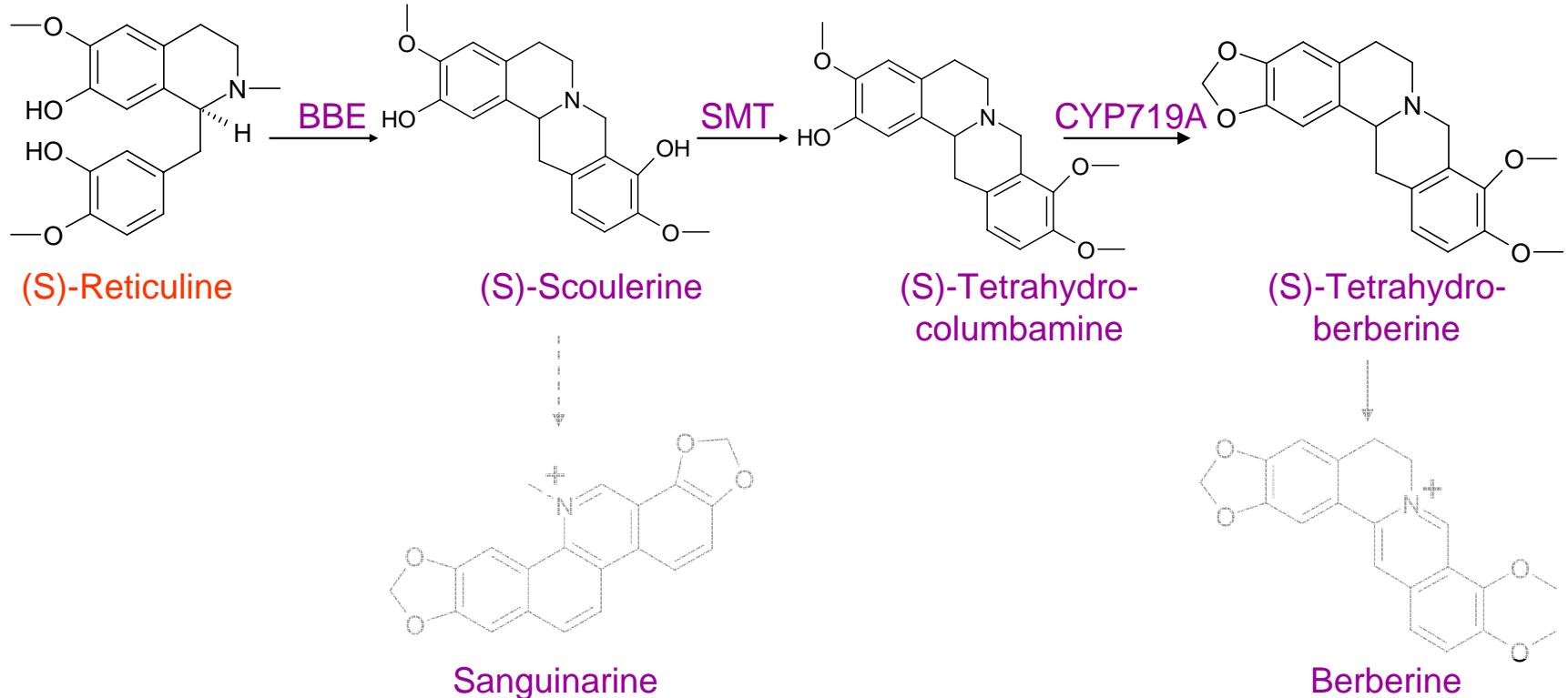
Optimization of (R,S)-Reticuline production



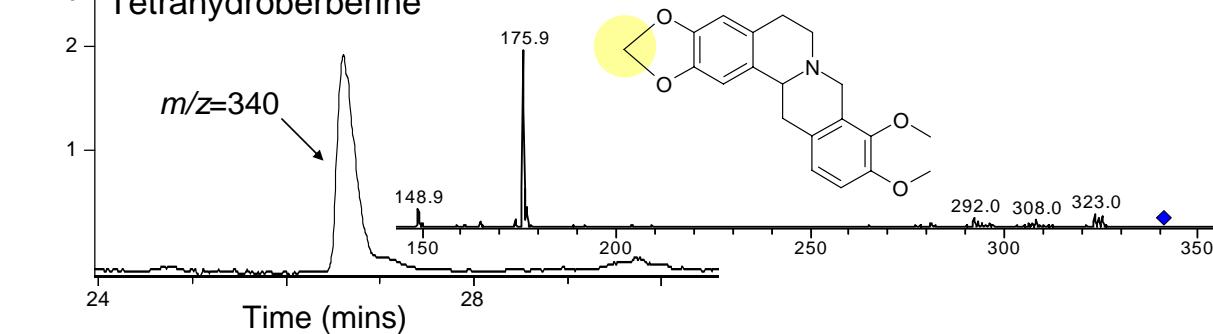
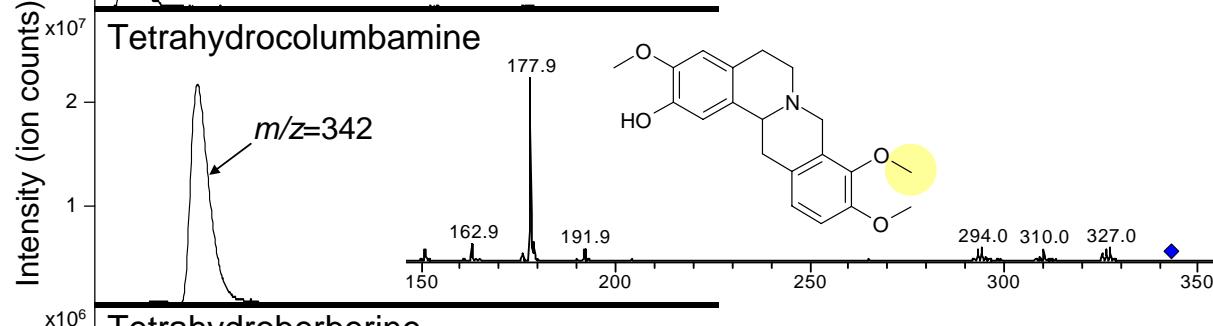
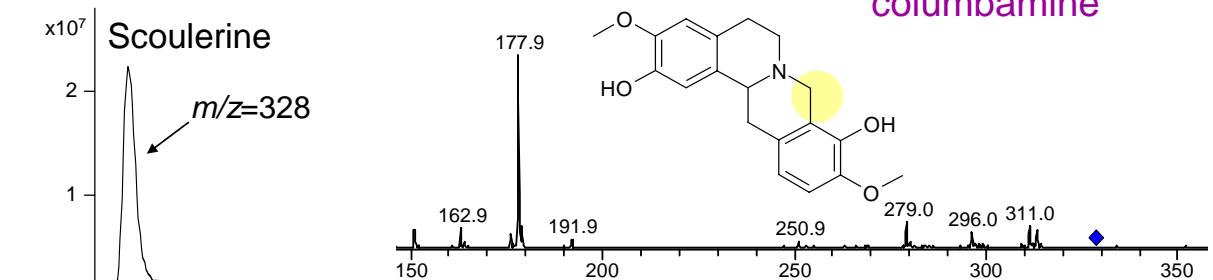
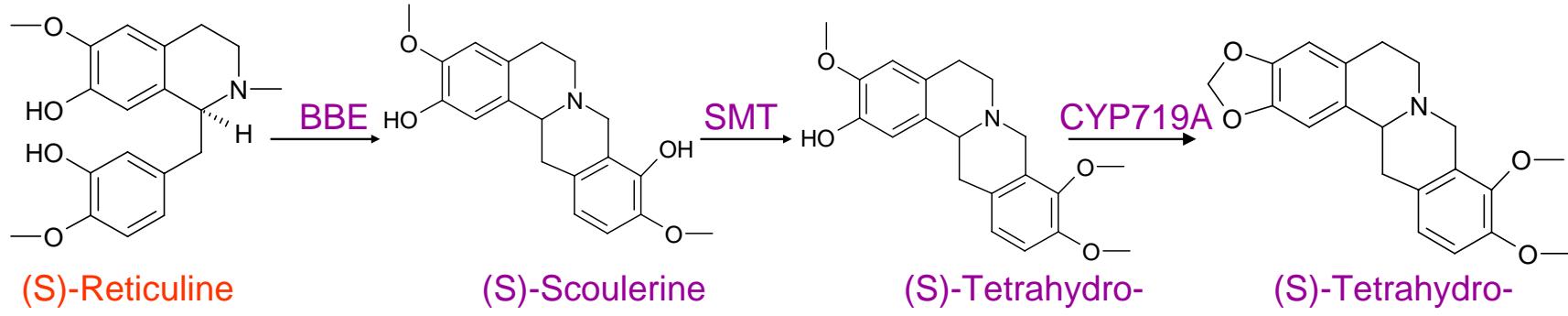
Enzyme level dependence:



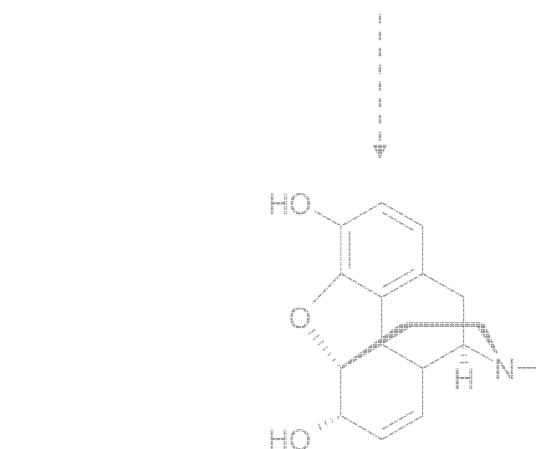
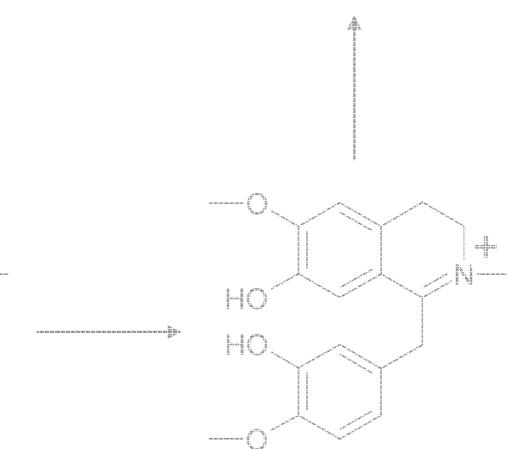
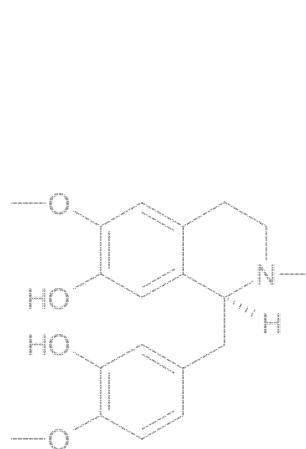
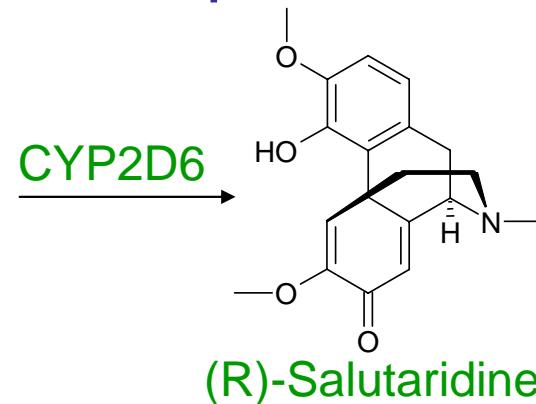
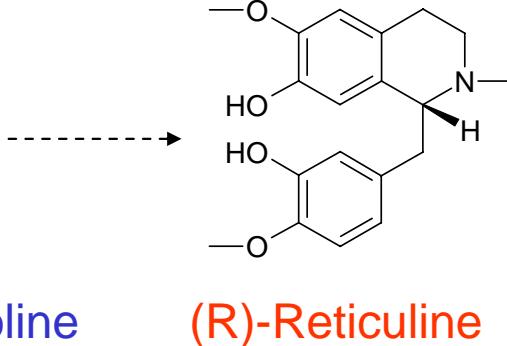
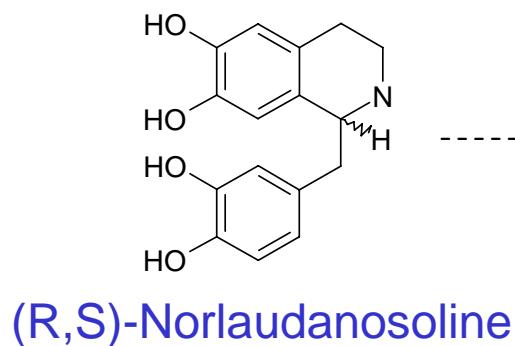
BIA synthesis beyond reticuline – berberine branch



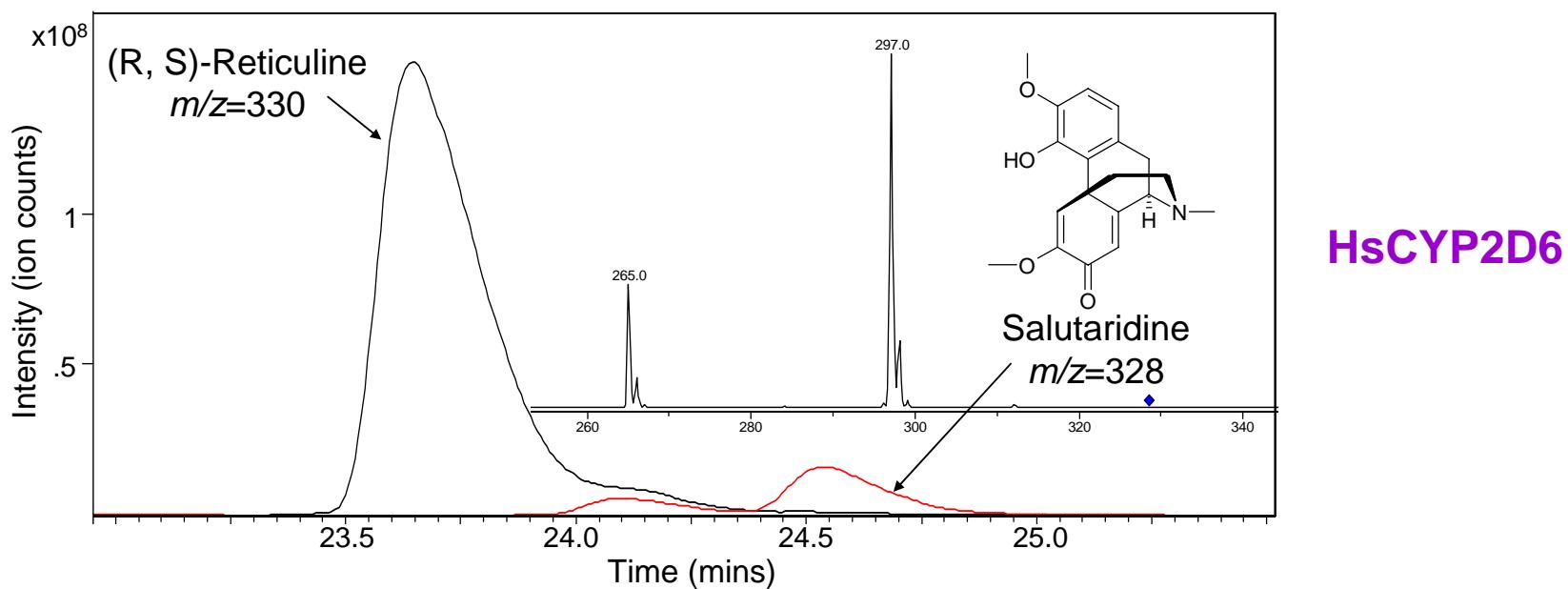
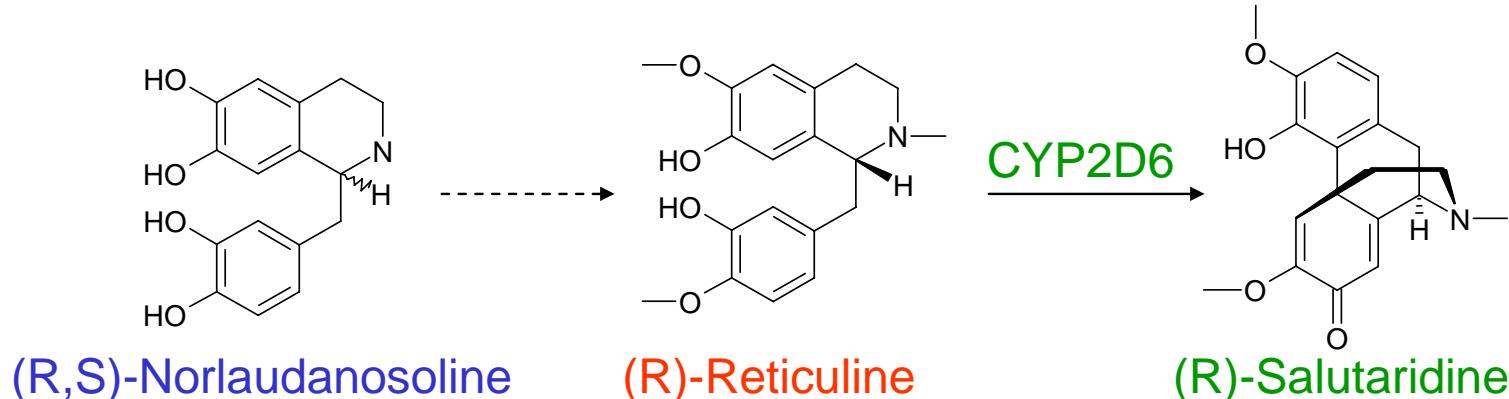
BIA synthesis beyond reticuline – berberine branch



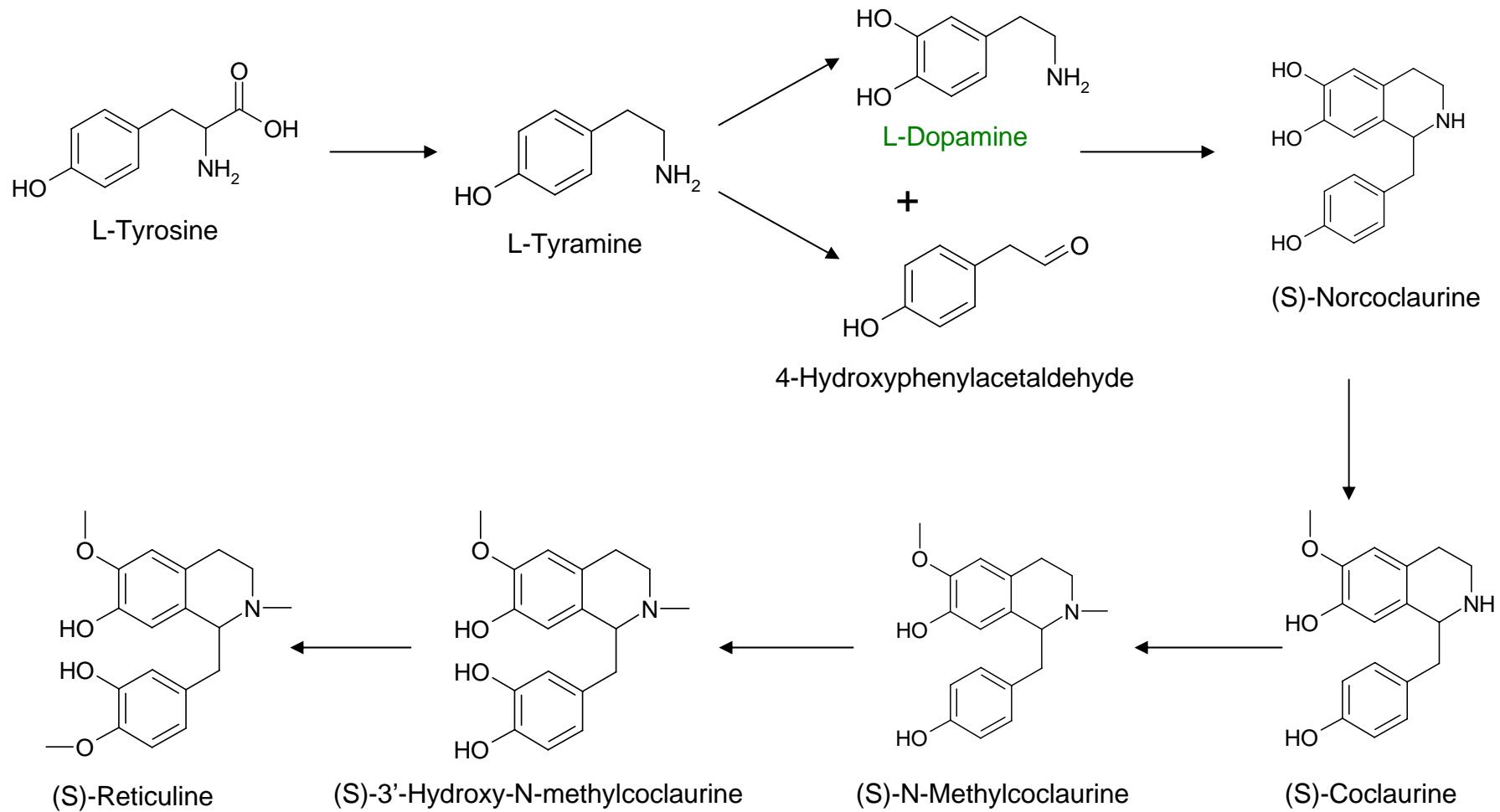
BIA synthesis beyond reticuline – morphine branch



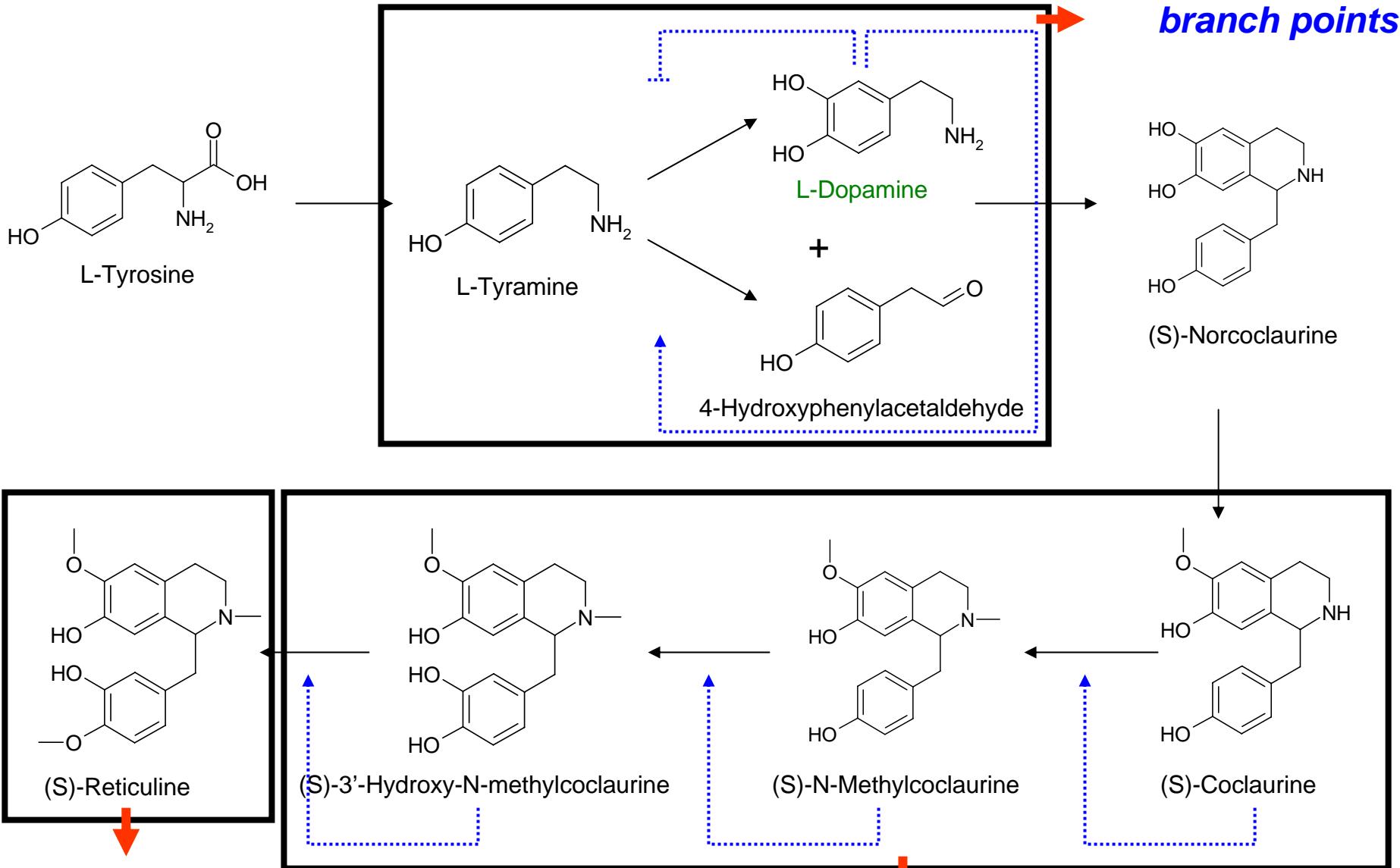
BIA synthesis beyond reticuline – morphine branch



Tools for optimizing BIA production



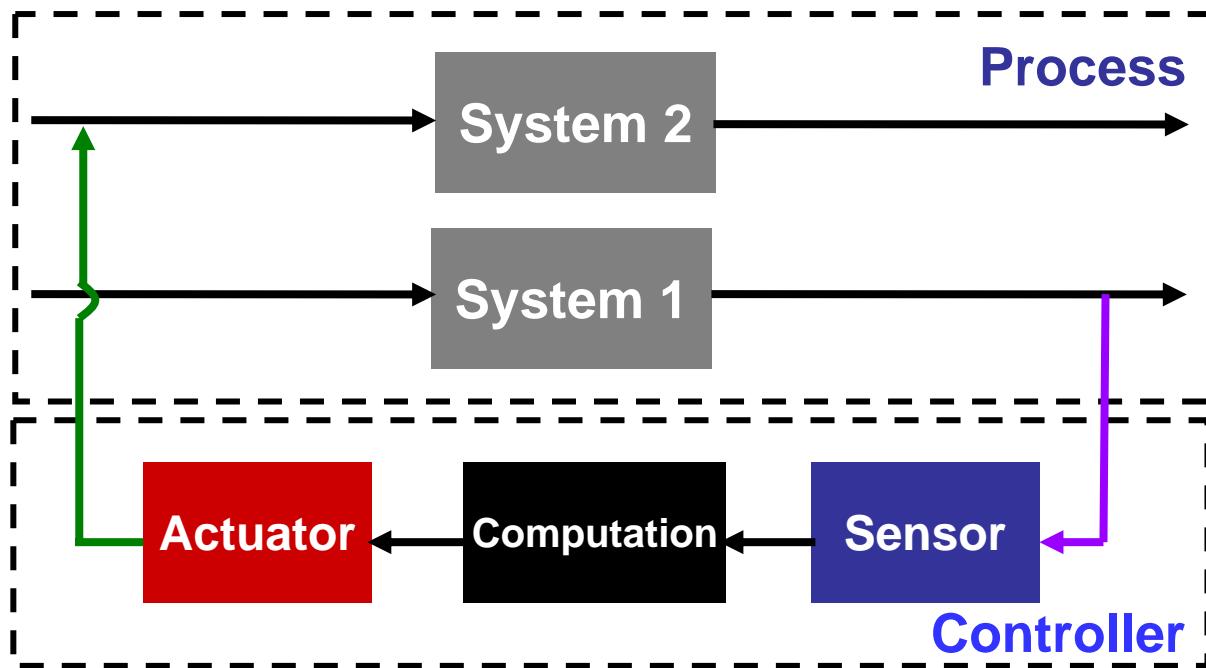
Tools for optimizing BIA production



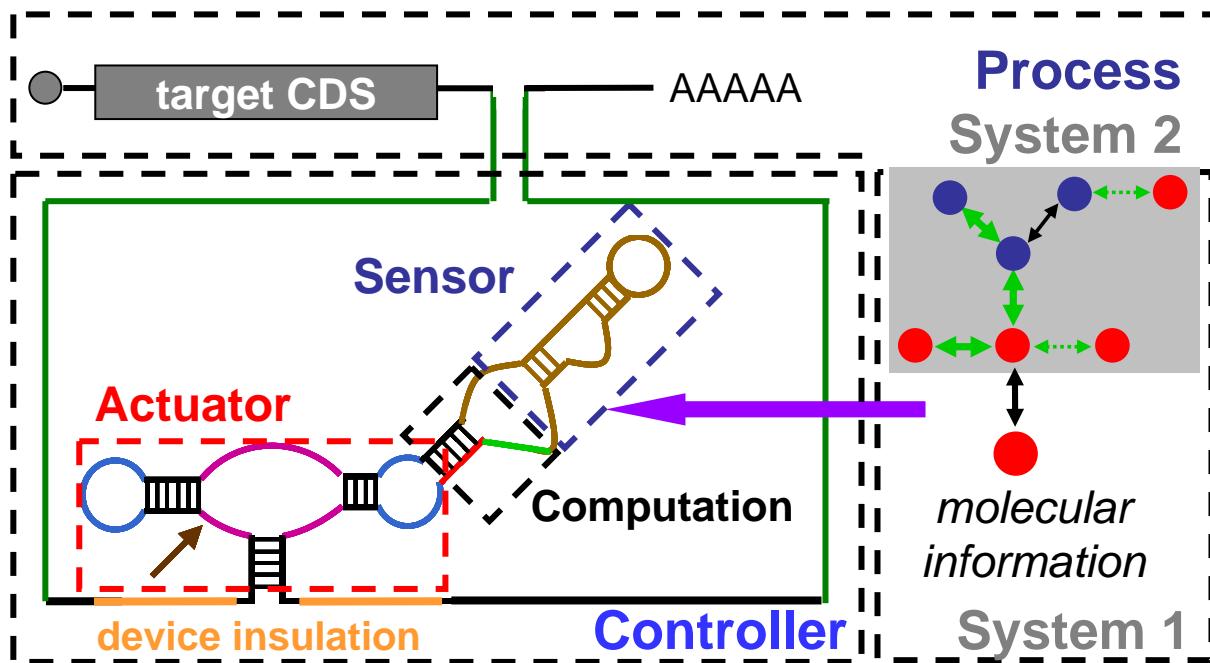
**Noninvasive sensing of
metabolite production**

Temporal regulation of enzyme cascades

General control system

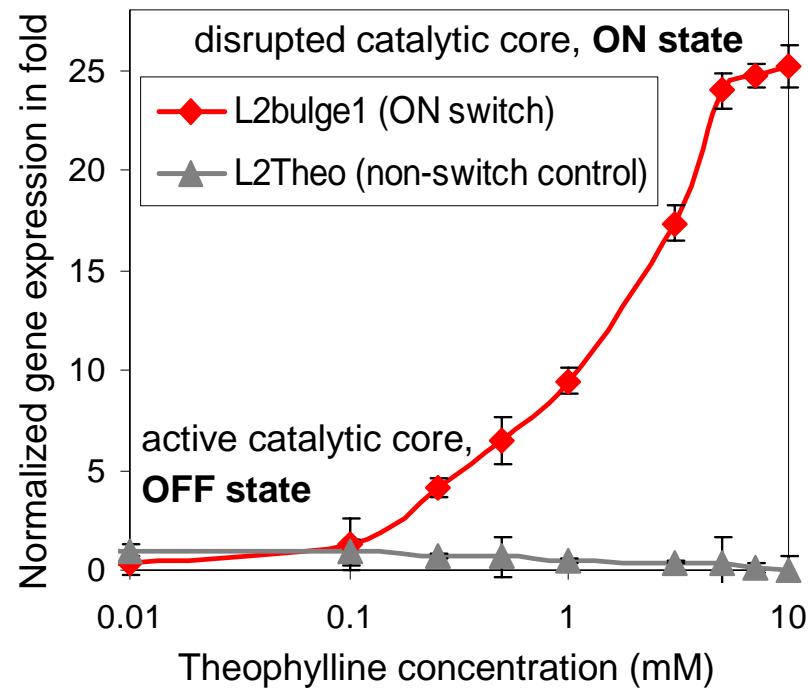
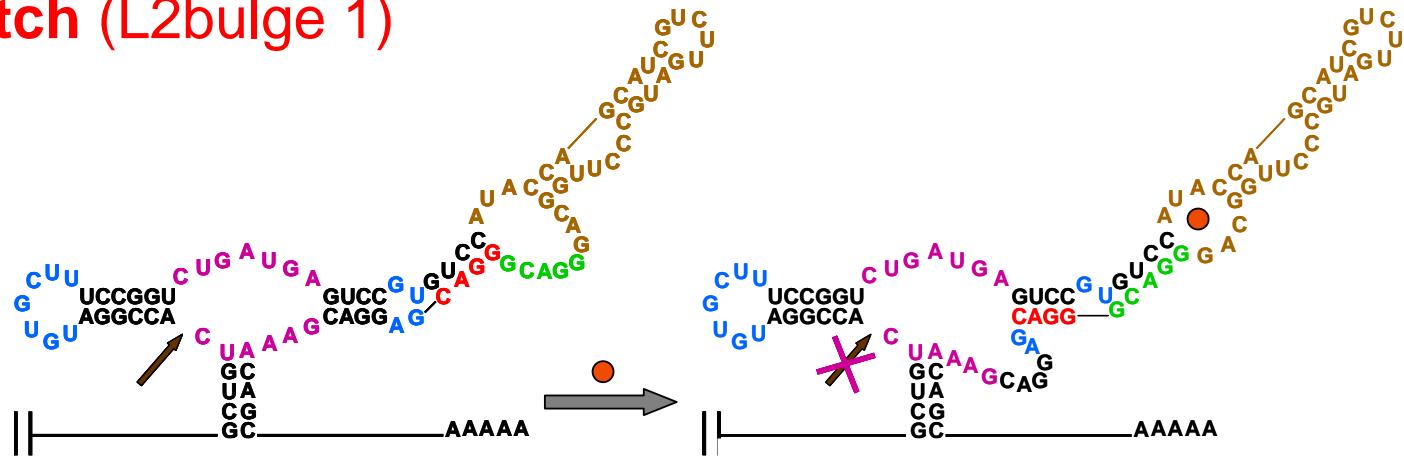


General biological control system



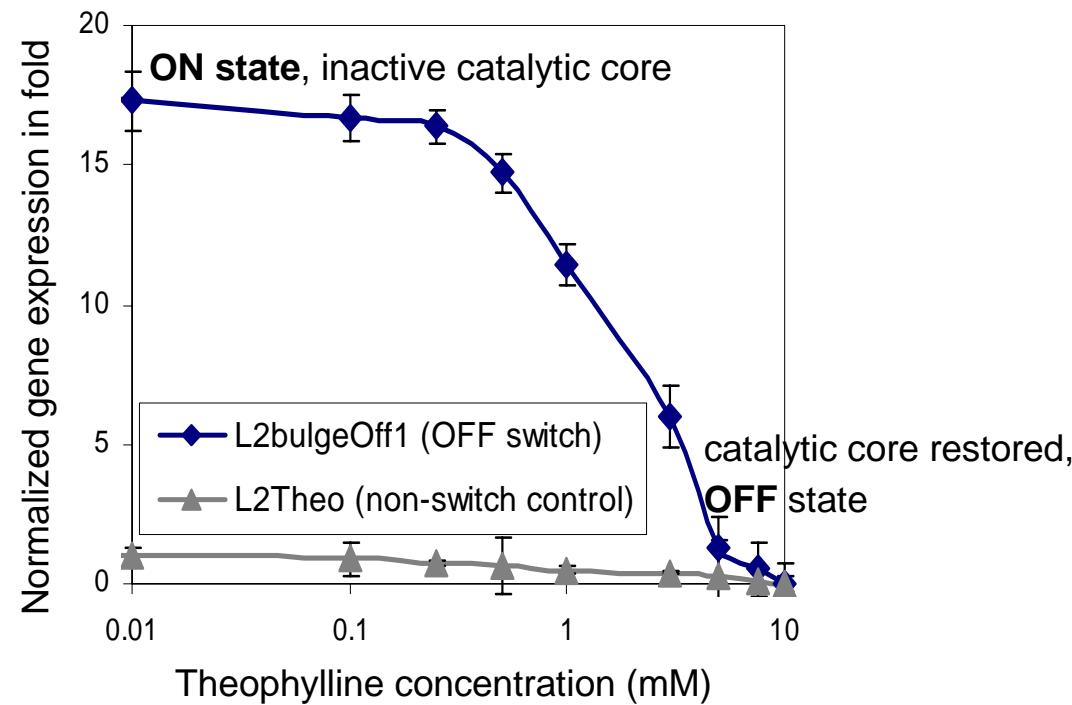
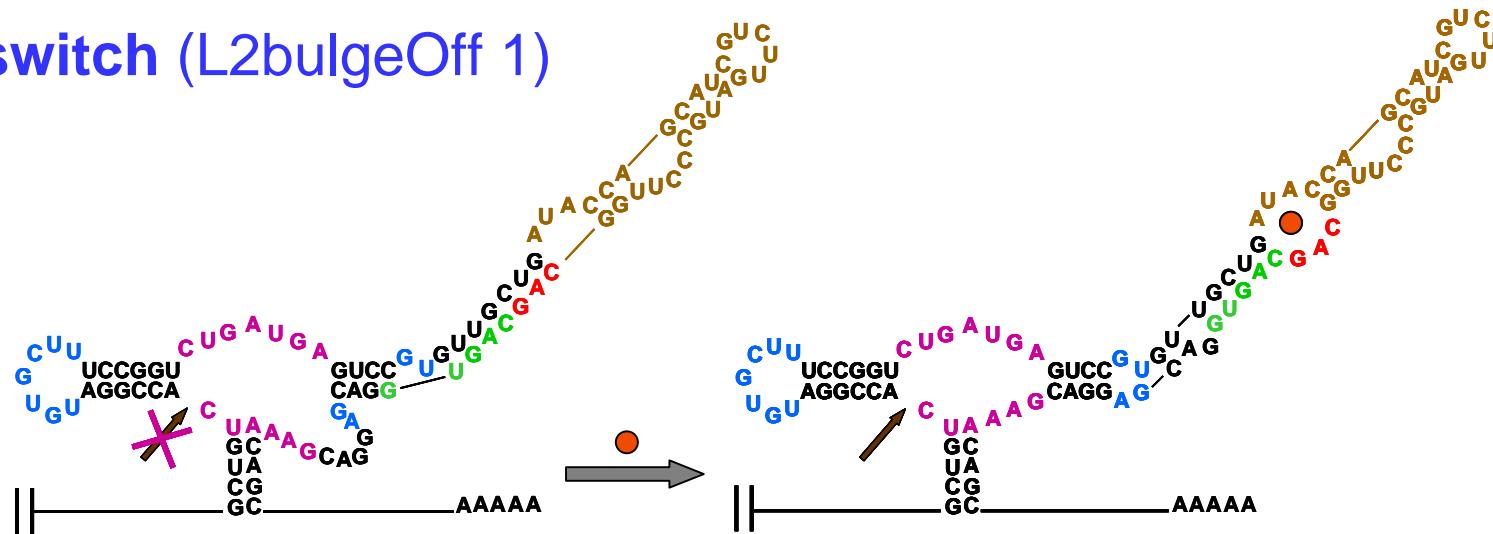
A ribozyme switch platform for up-regulating expression

ON switch (L2bulge 1)

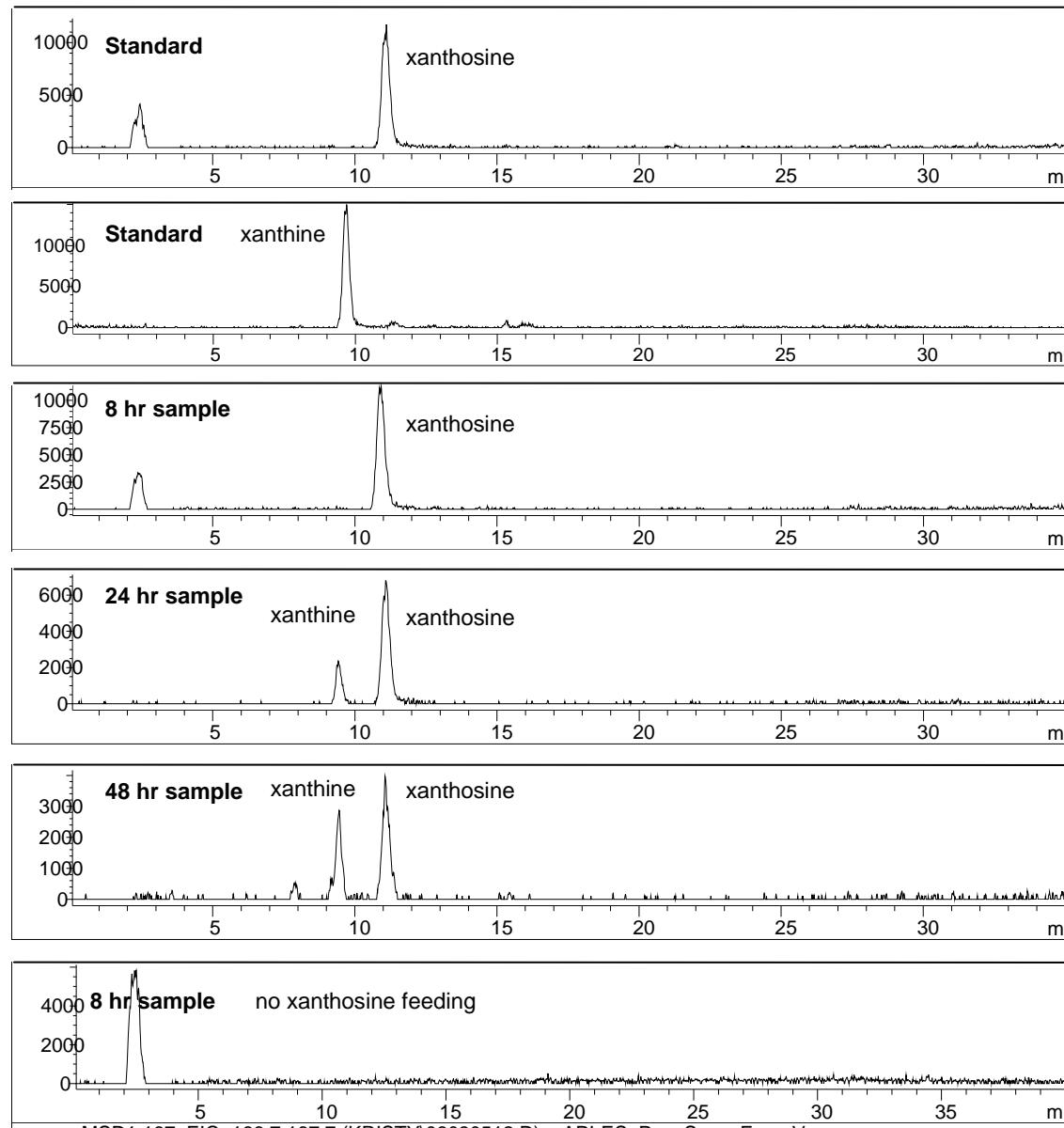
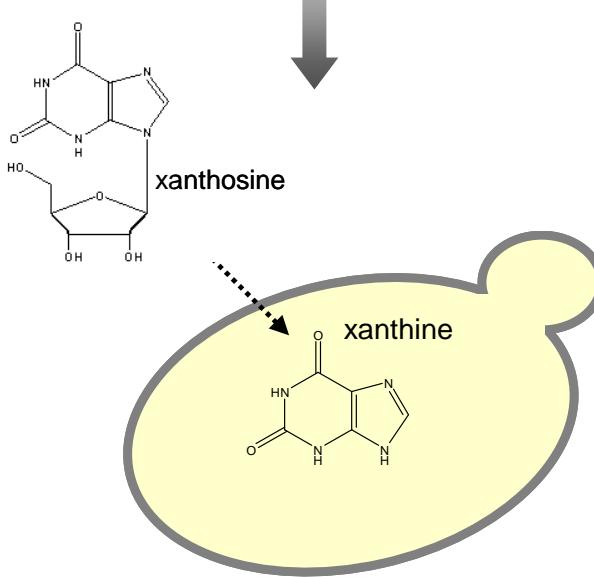
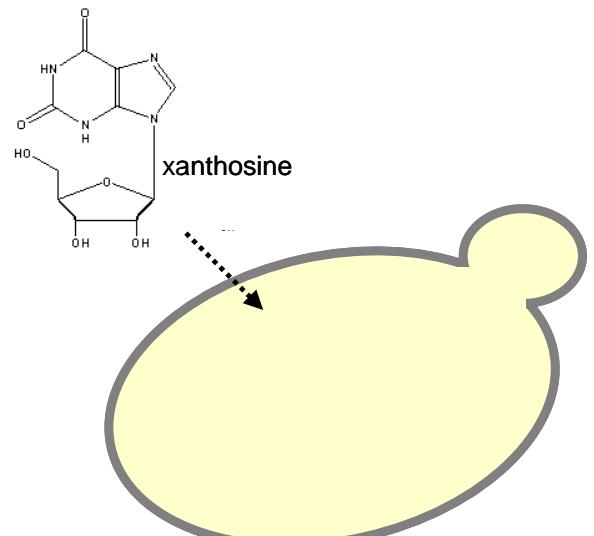


A ribozyme switch platform for down-regulating expression

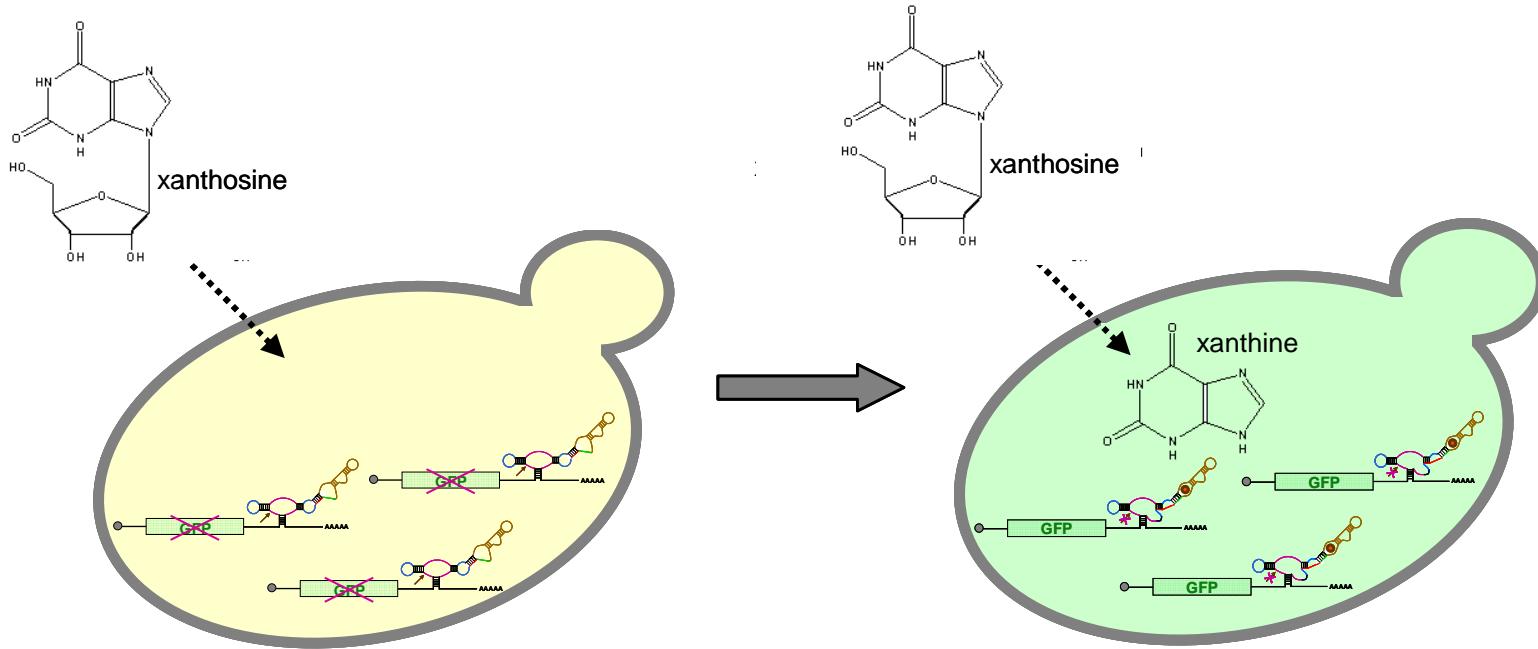
OFF switch (L2bulgeOff 1)



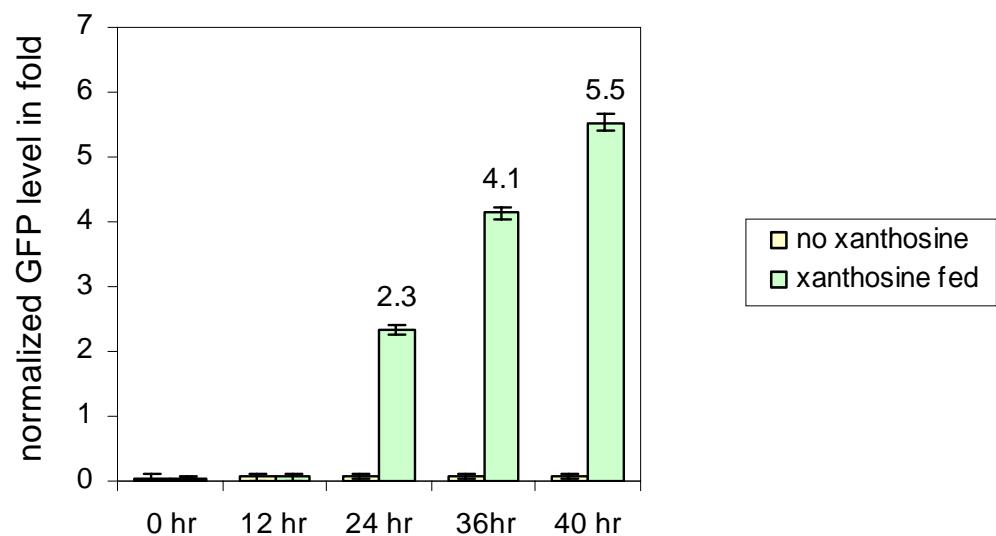
Integrating RNA devices as noninvasive sensors of metabolite concentration



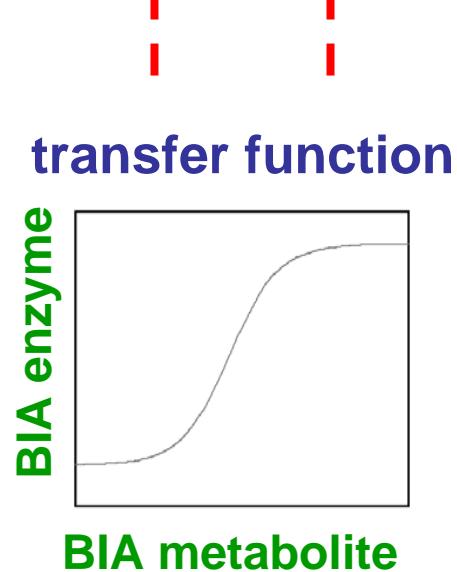
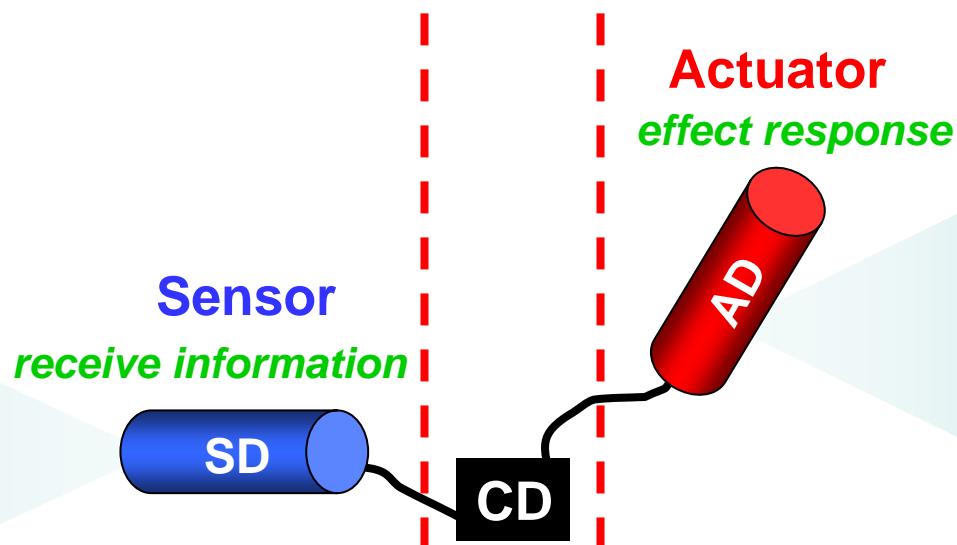
Integrating RNA devices as noninvasive sensors of metabolite concentration



Implementation of ON ribozyme switch as an real-time noninvasive xanthine sensor



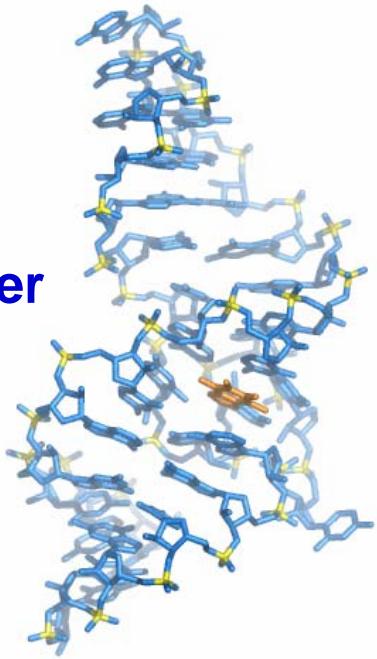
Small molecules
Proteins
RNA
DNA
Metal ions
Temperature
pH



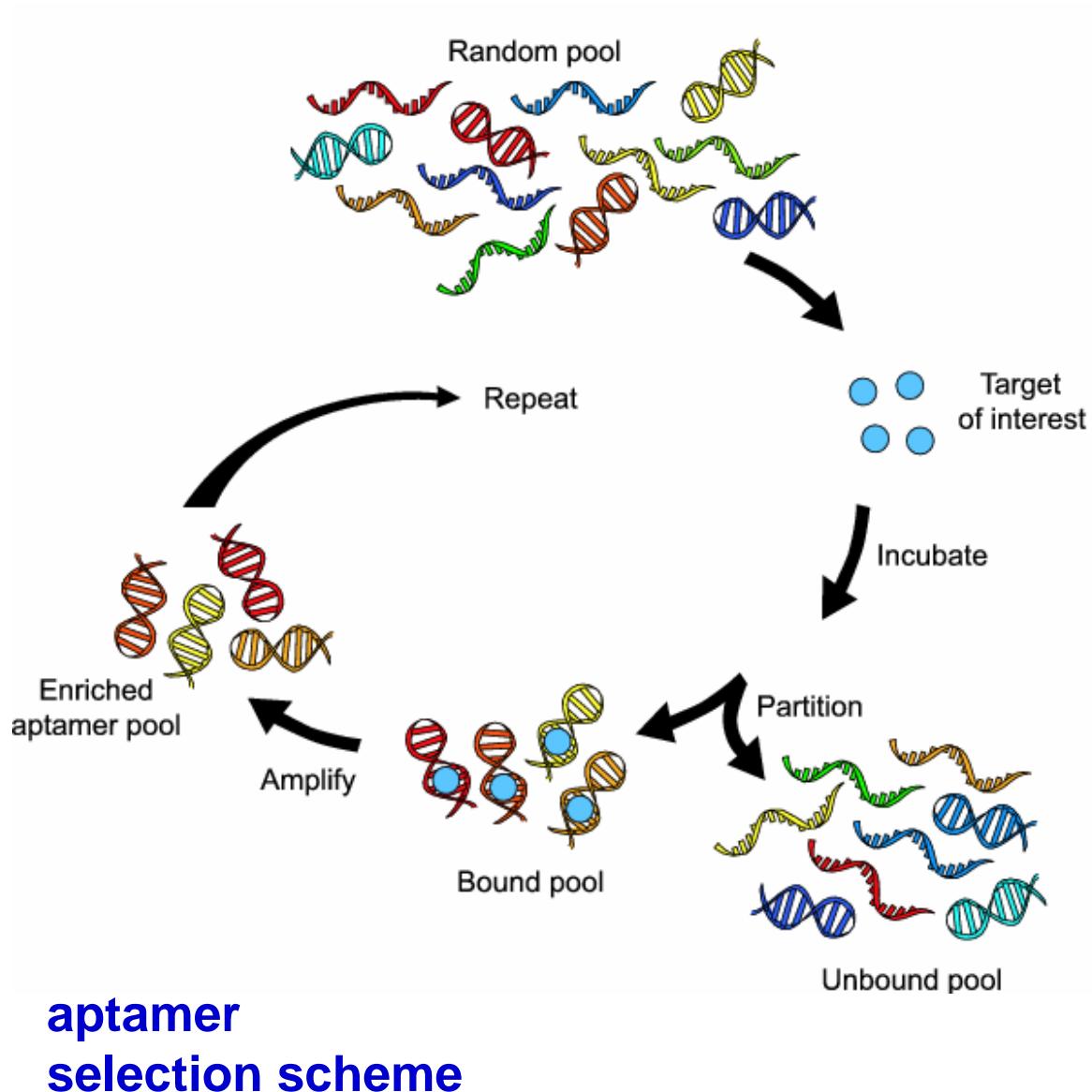
Transcription
Translation
Degradation
Splicing
Enzyme activity
Complex formation

Scalability challenge: libraries of nucleic acid sensors

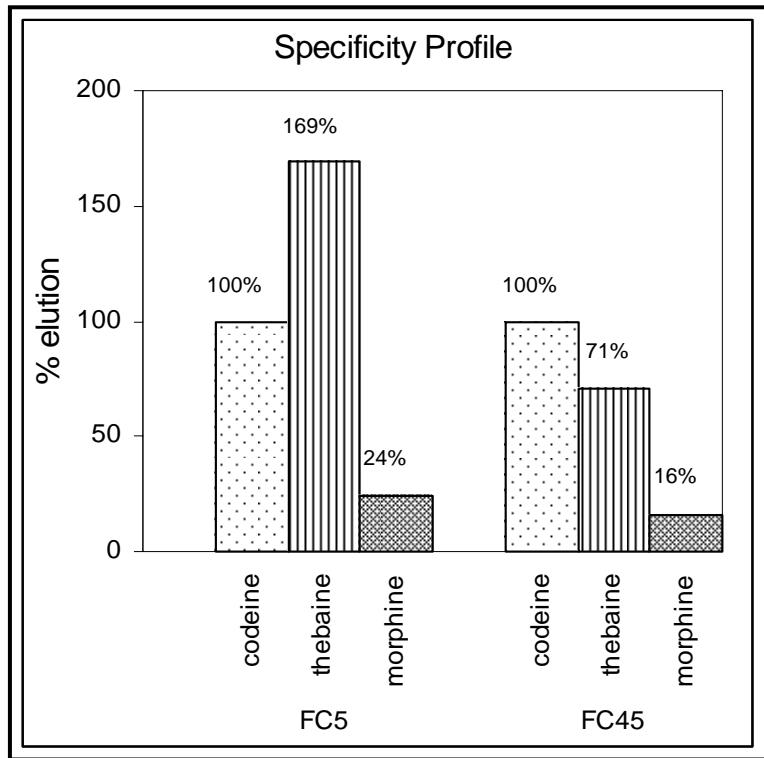
aptamer



- bind wide range of ligands
- high specificity and affinity
- generated through *in vitro* selection process



Specificities of BIA-binding aptamers



FC5

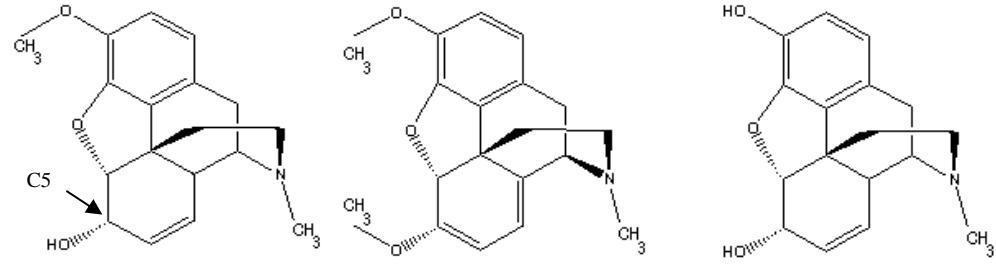
5' A G G A U U
U G A C G G
G A C • G G
C • U G A
A G G G
U • G G
C • G A
G G A
G A • U
C • G G
A • U G
G • C G
G • C G
G 3'
3'

FC45

5' G G G C C 3'
G U G A G A
U A G C U G G A A
A G U G C • G G A A
A G U G C • G G U
C U G G G G A A
G G G C C 3'

$$K_d (\text{FC5L}) = 4.55 \pm 0.14 \mu\text{M}$$

$$K_d (\text{FC45L}) = 2.59 \pm 0.09 \mu\text{M}$$



High-throughput SPR-based characterization strategy enables rapid screening for mini-aptamer sequences and structural analysis

Integrating synthetic metabolic networks and RNA-based control systems

- Metabolic pathway engineering requires a host of tools for optimizing flux and product accumulation
- User-programmed feedback control systems are useful for dynamically controlling flux through pathways
- Developing new genetically encoded tools for receiving, processing, and transmitting molecular information
- Response properties can be programmed to fit the performance specifications of a given application
- These technologies will advance the engineering of more robust cellular systems

Acknowledgements

The Smolke Lab

Postdoctoral Researchers

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Kristy Hawkins

Joe Liang

Josh Michener

Michael Siddiqui

Jay Vowles

Maung Nyan Win

Technicians

Midori Greenwood-Goodwin

Collaborators

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The Jensen Lab (COH)



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Department of Defense (BCRP)

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Engineering for Medicine (Caltech)

National Institutes of Health (NCI)

National Institutes of Health (NIGMS)

National Science Foundation (BES)