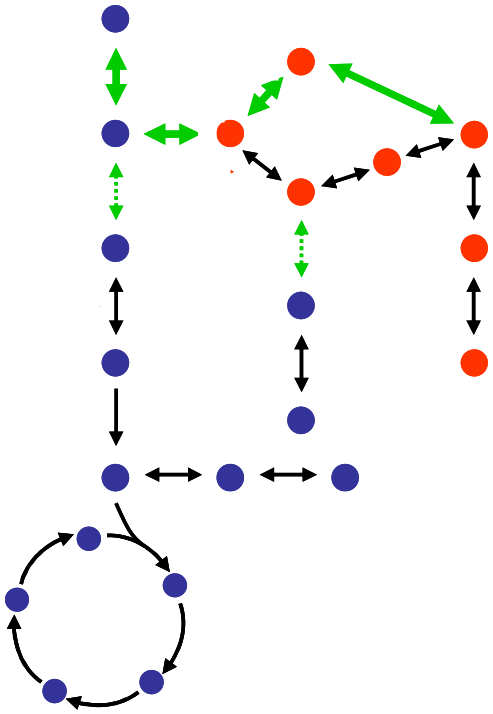
Foundational advances in RNA engineering applied to control of biosynthesis

Christina D. Smolke
Division of Chemistry and Chemical Engineering
California Institute of Technology

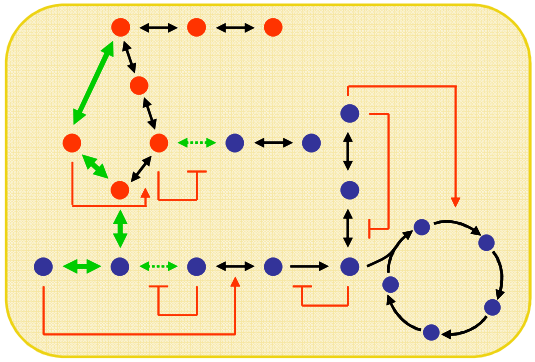
February 11, 2008
MEWG: Interagency Conference on Metabolic Engineering

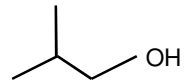
The role of feedback control in biosynthesis



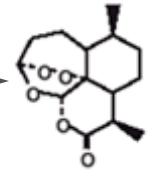
Applications of engineered biological systems

Metabolic engineering

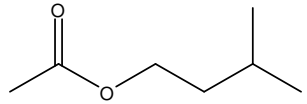




Isobutanol
biofuels



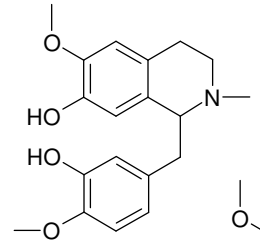
Artemisinin
drugs



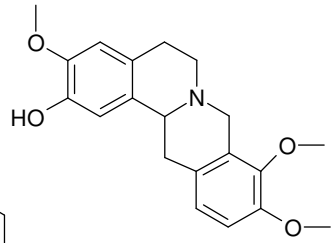
Isoamyl acetate
scents, flavors

Benzylisoquinoline alkaloid biosynthesis

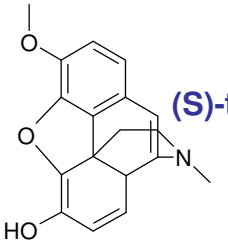
Papaver somniferum (opium poppy)



(S)-reticuline



(S)-tetrahydrocolumbamine

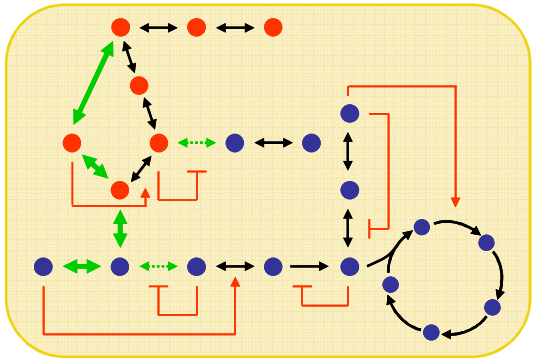


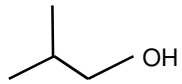
codeine

http://www.nature.com/news/2004/040816/images/yeast.jpg
http://www.westwindphotos.ca/images/101%20Opium%20Poppy.jpg

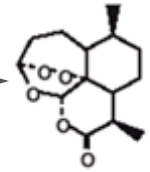
Applications of engineered biological systems

Metabolic engineering

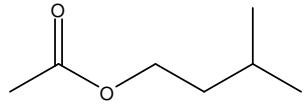




Isobutanol
biofuels

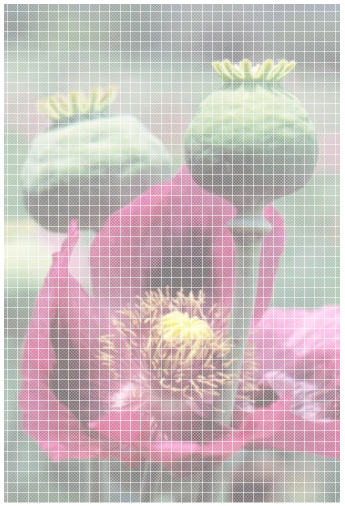


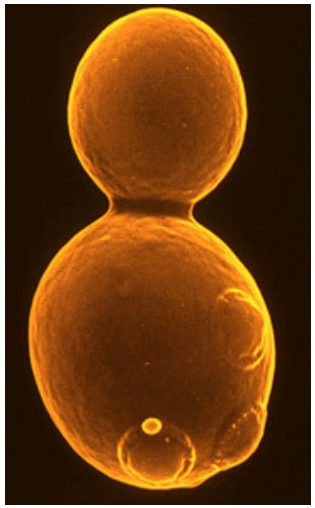
Artemisinin
drugs

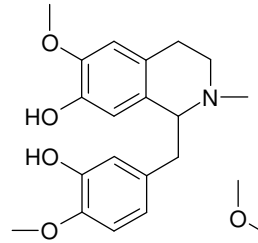


Isoamyl acetate
scents, flavors

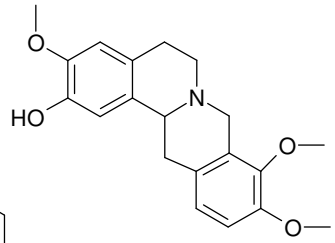
Benzylisoquinoline alkaloid microbial synthesis



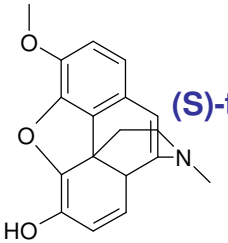




(S)-reticuline



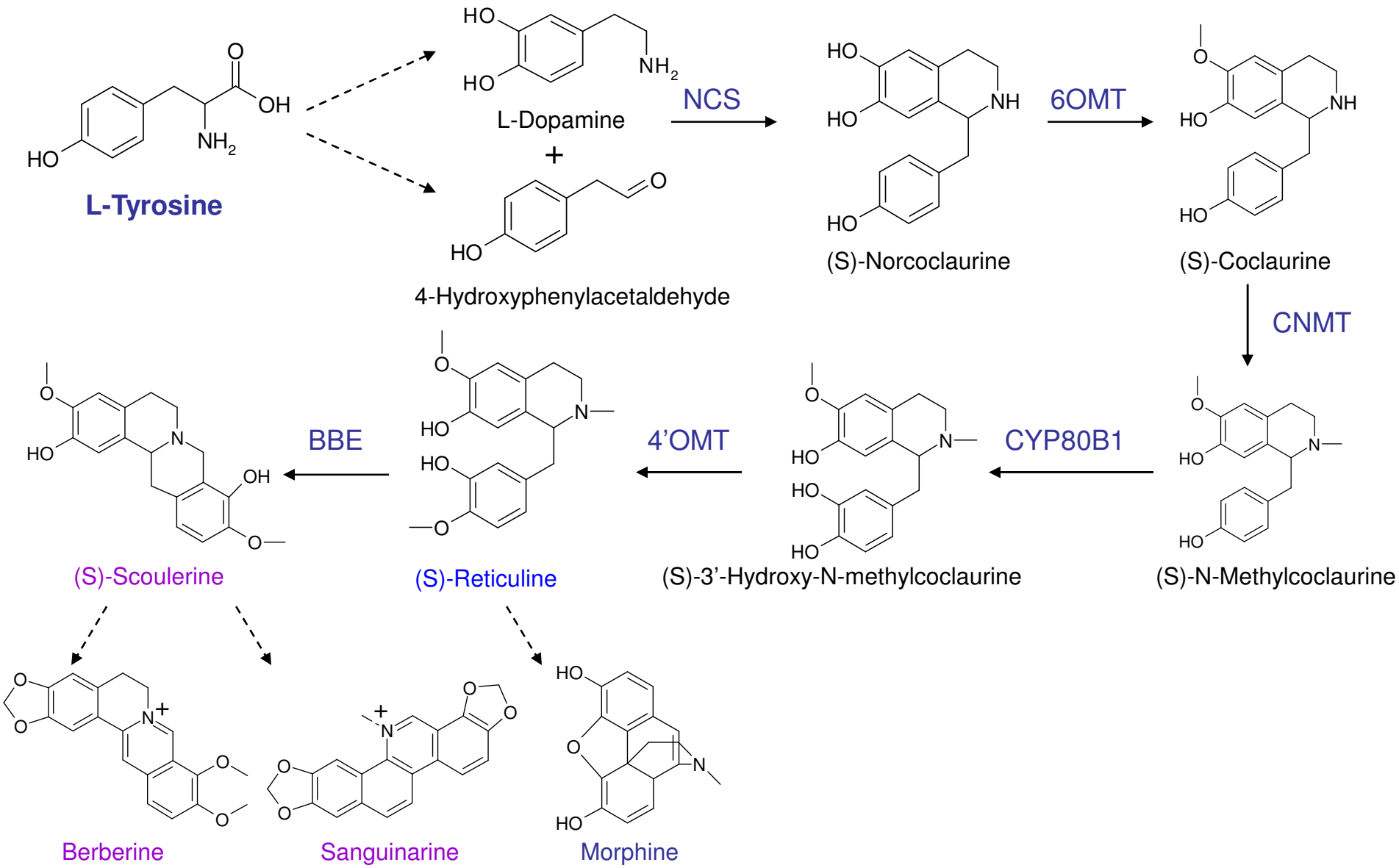
(S)-tetrahydrocolumbamine



codeine

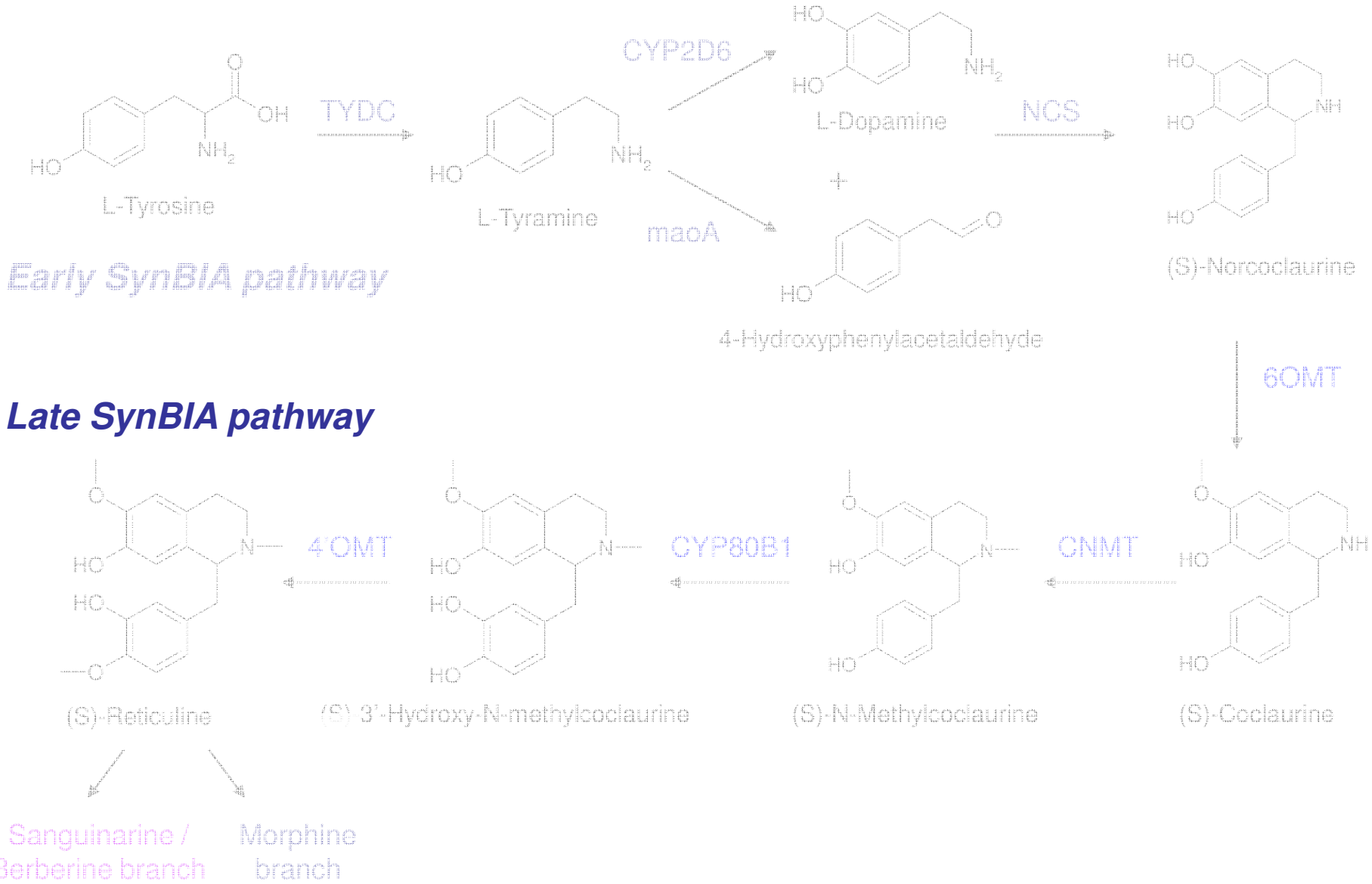
http://www.nature.com/news/2004/040816/images/yeast.jpg
http://www.westwindphotos.ca/images/101%20Opium%20Poppy.jpg

Native BIA pathway



Facchini PJ. 2001. *Annu Rev Plant Physiol Plant Mol Biol.* 52: 29-66

Synthetic BIA pathway

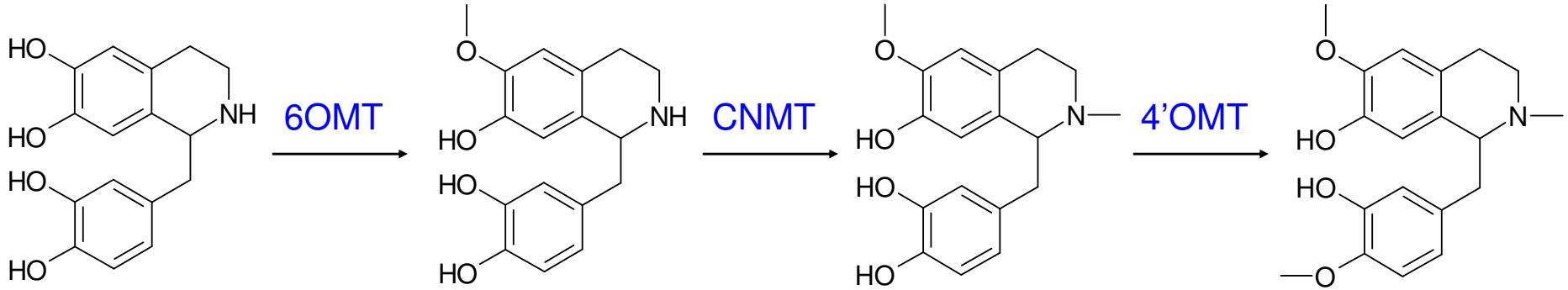


Early SynBIA pathway

Late SynBIA pathway

Hawkins KH, Smolke CD. 2007. *In preparation.*

Late Synthetic BIA pathway



(R,S)-Norlaudanosoline

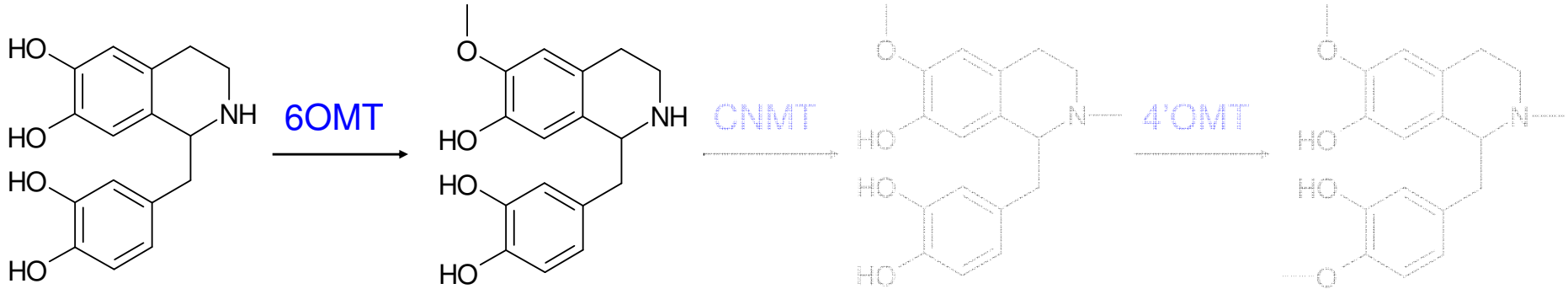
(R,S)-6-*O*-Methyl-norlaudanosoline

(R,S)-3'-Hydroxy-*N*-methylcoclaurine

(R,S)-Reticuline

Peter Facchini, U. Calgary
Ounaroon A, et al. 2003. *Plant J.* 36: 808-19

Production of 6-*O*-Methyl-norlaudanosoline

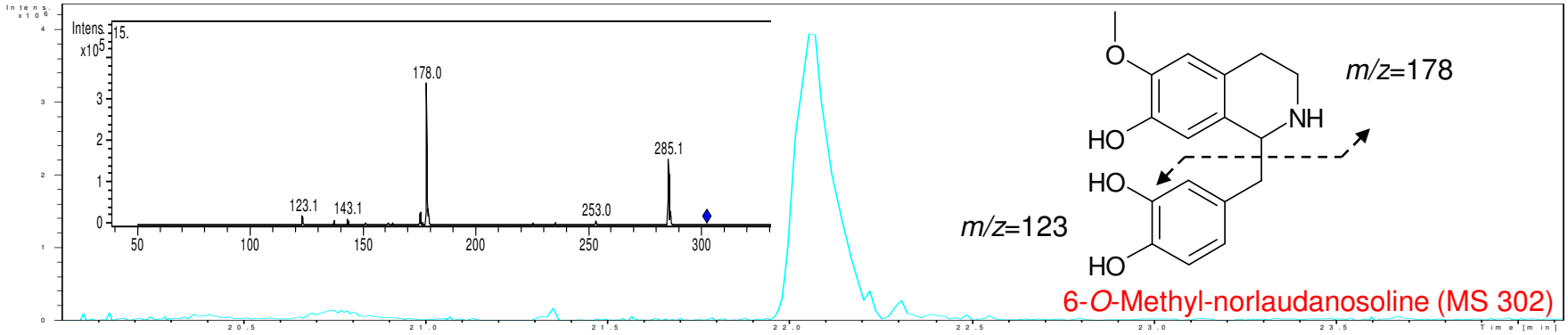


(R,S)-Norlaudanosoline

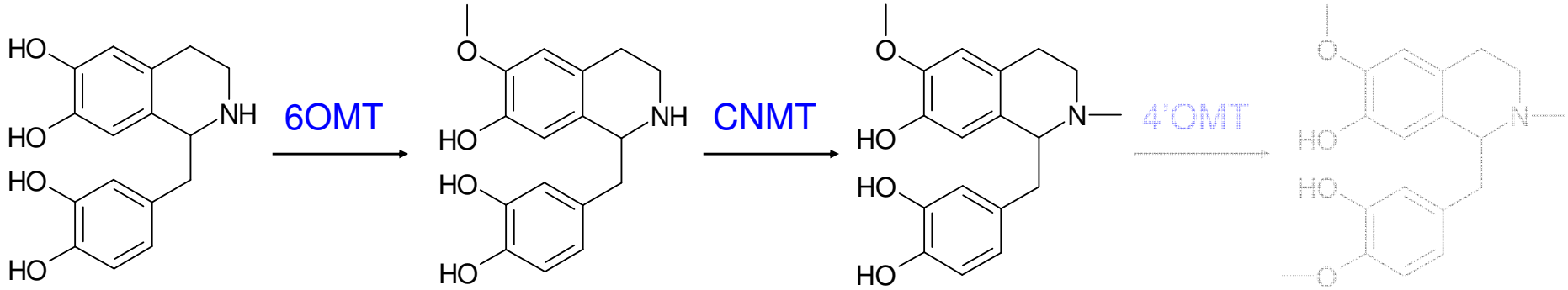
(R,S)-6-*O*-Methyl-norlaudanosoline

(R,S)-3'-Hydroxy-*N*-methylcoclaurine

(R,S)-Reticuline



Production of 3'-Hydroxy-*N*-methylcoclaurine

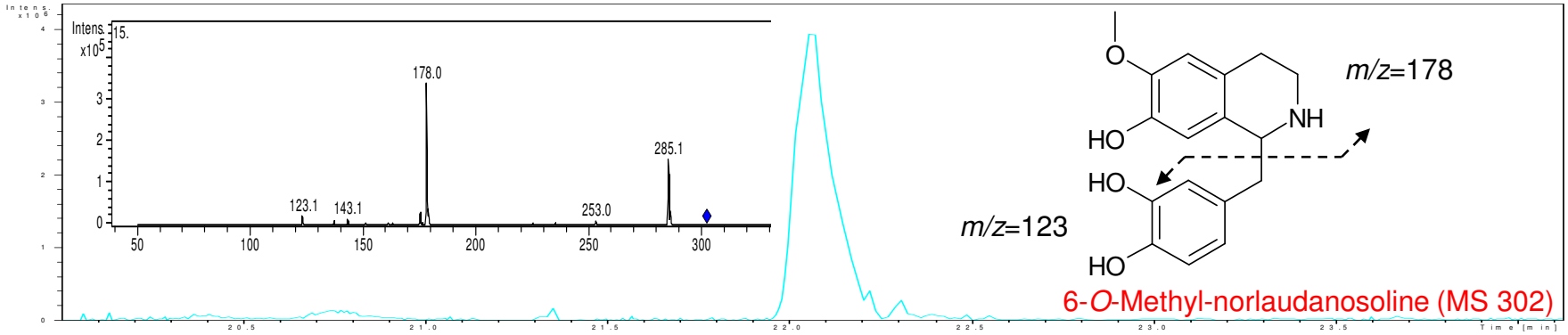


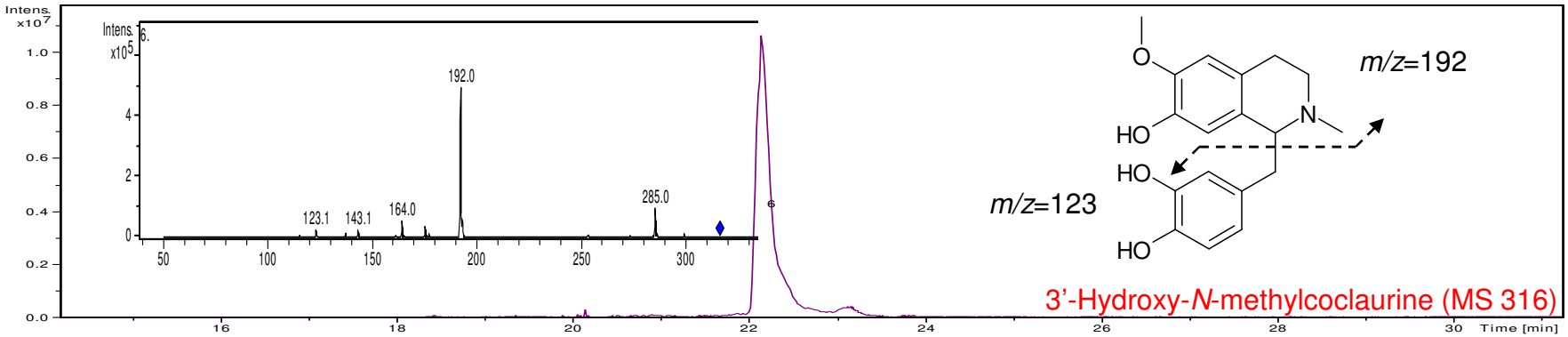
(R,S)-Norlaudanosoline

(R,S)-6-*O*-Methyl-norlaudanosoline

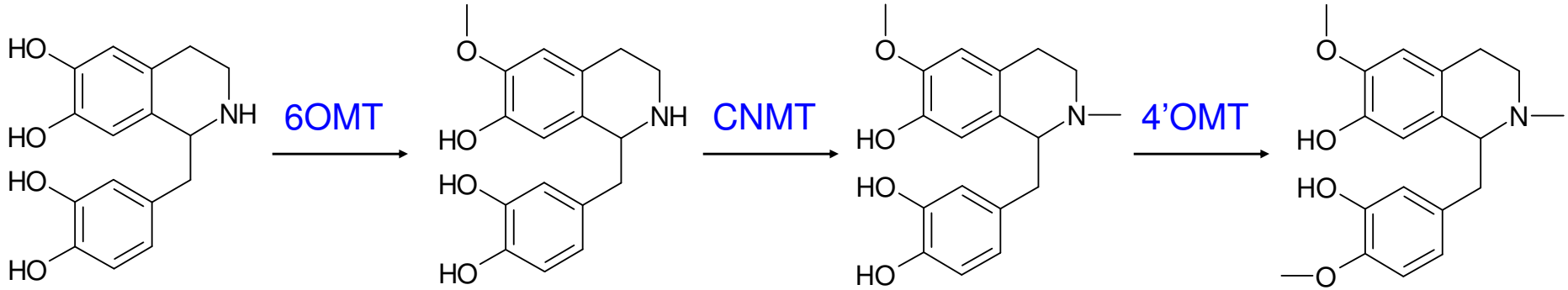
(R,S)-3'-Hydroxy-*N*-methylcoclaurine

(R,S)-Reticuline





Production of (R,S)-Reticuline

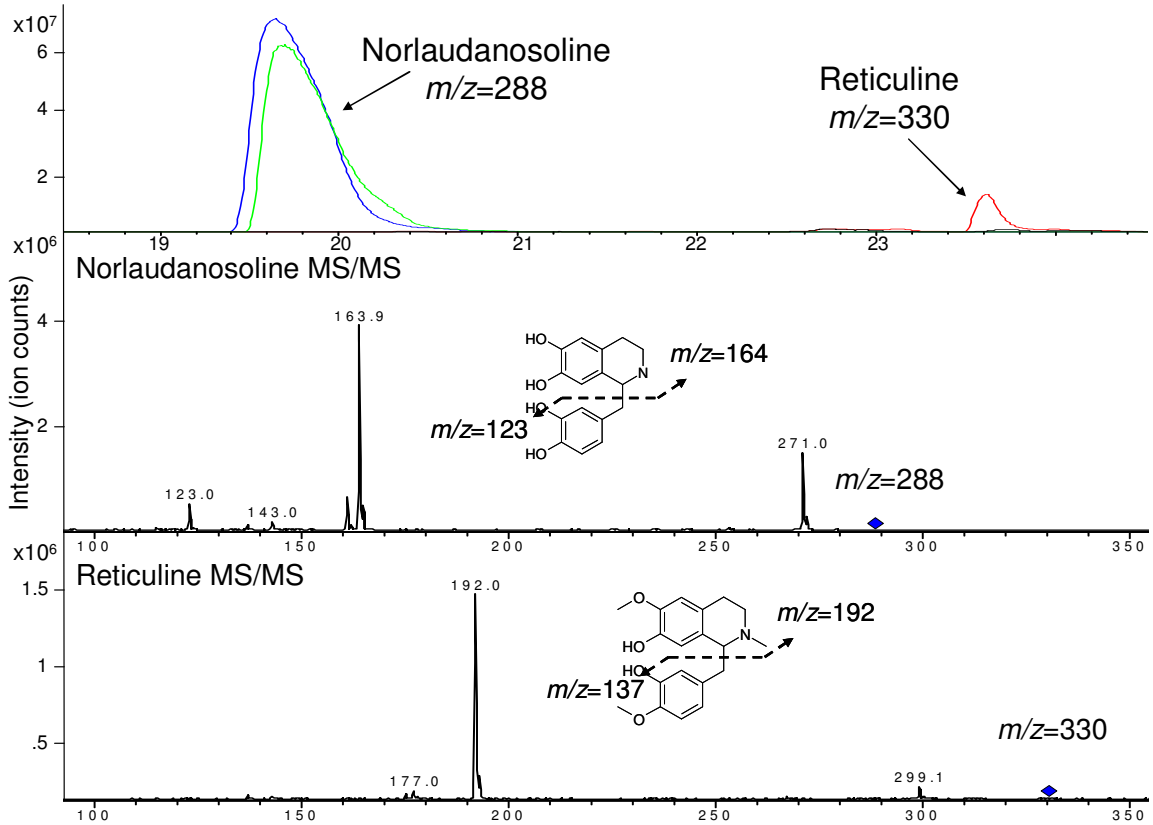


(R,S)-Norlaudanosoline

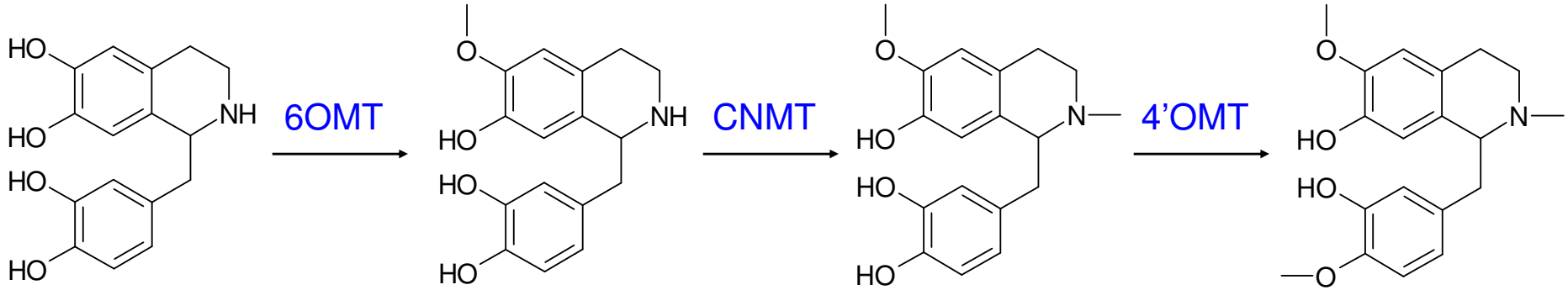
(R,S)-6-*O*-Methyl-norlaudanosoline

(R,S)-3'-Hydroxy-*N*-methylcoclaurine

(R,S)-Reticuline



Optimization of (R,S)-Reticuline production



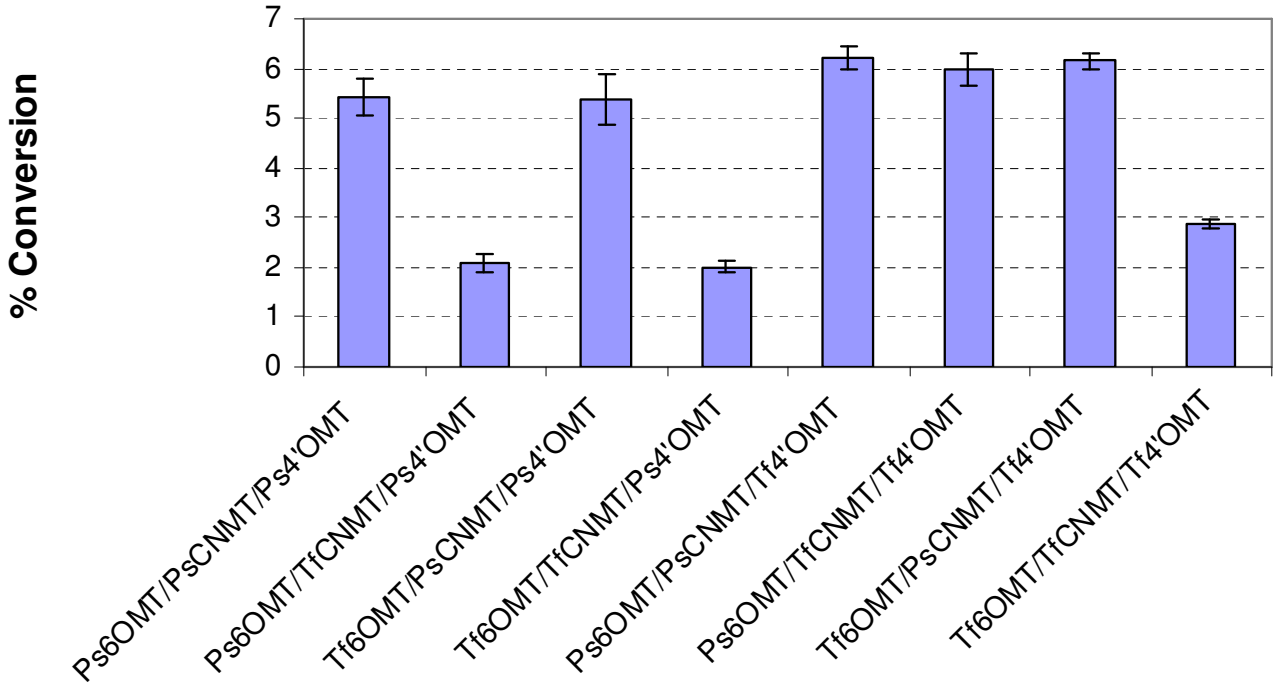
(R,S)-Norlaudanosoline

(R,S)-6-*O*-Methyl-norlaudanosoline

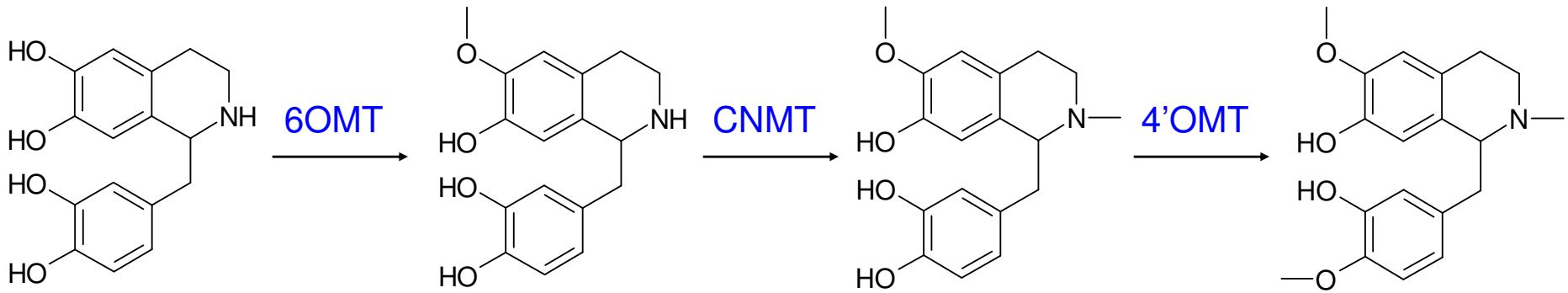
(R,S)-3'-Hydroxy-*N*-methylcoclaurine

(R,S)-Reticuline

Enzyme variant dependence:



Optimization of (R,S)-Reticuline production



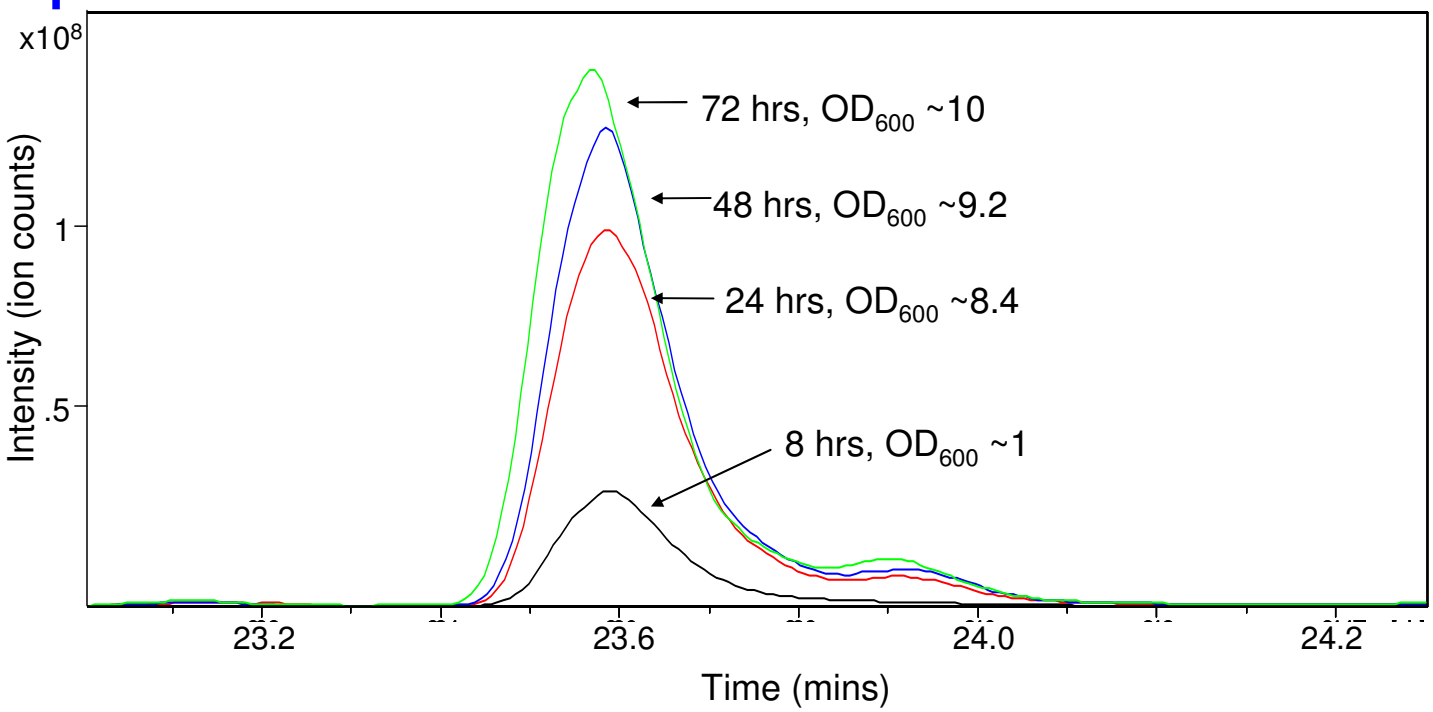
(R,S)-Norlaudanosoline

(R,S)-6-*O*-Methyl-norlaudanosoline

(R,S)-3'-Hydroxy-*N*-methylcoclaurine

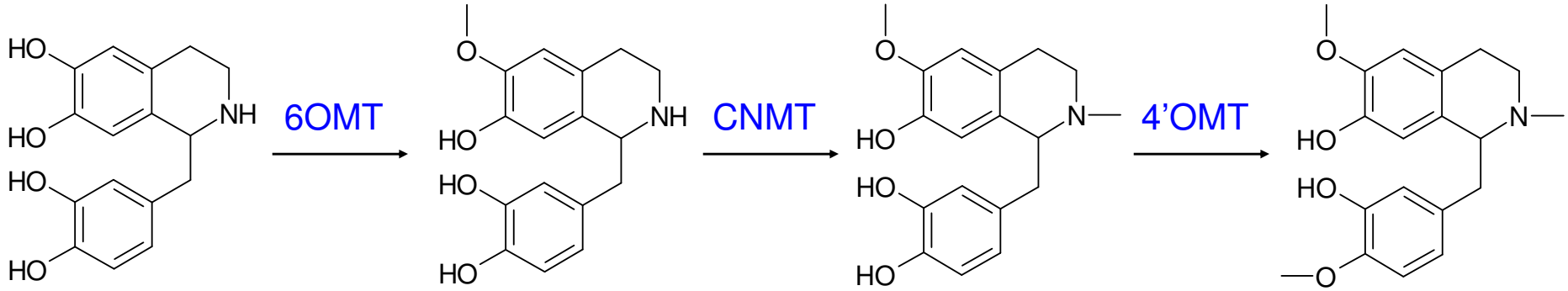
(R,S)-Reticuline

Time dependence:



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

Optimization of (R,S)-Reticuline production



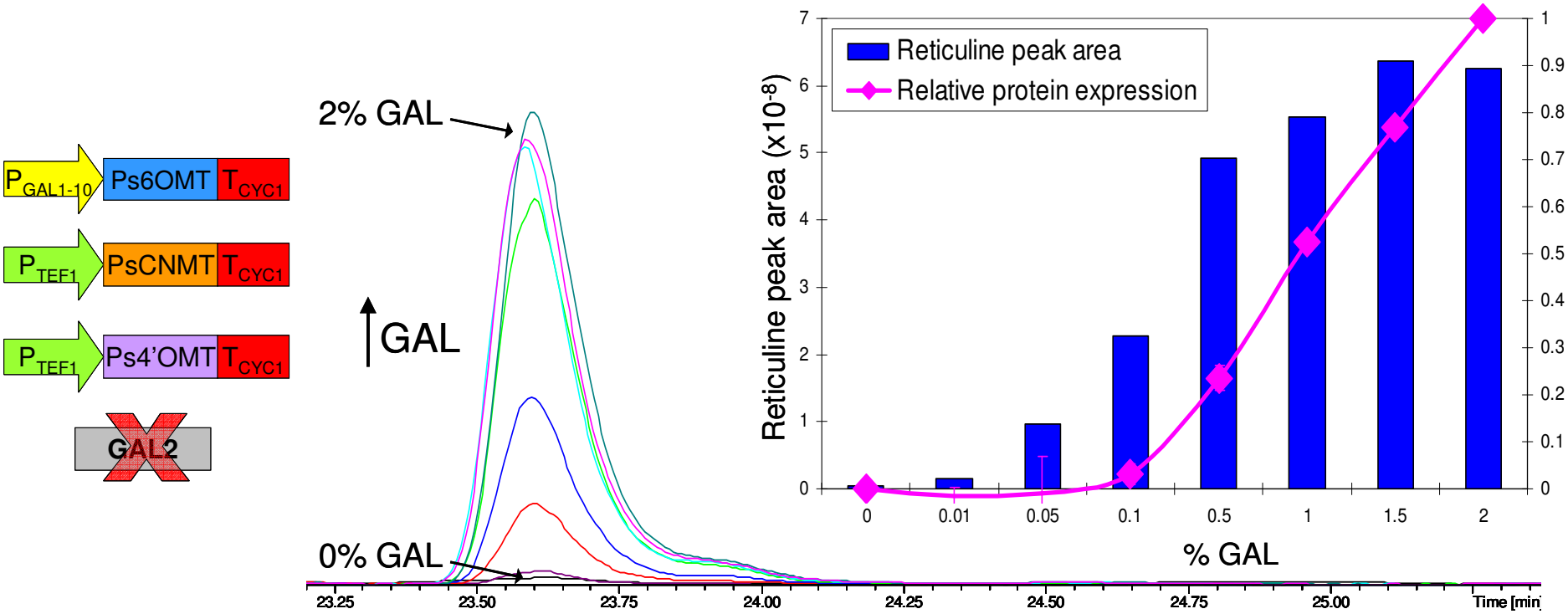
(R,S)-Norlaudanosoline

(R,S)-6-*O*-Methyl-norlaudanosoline

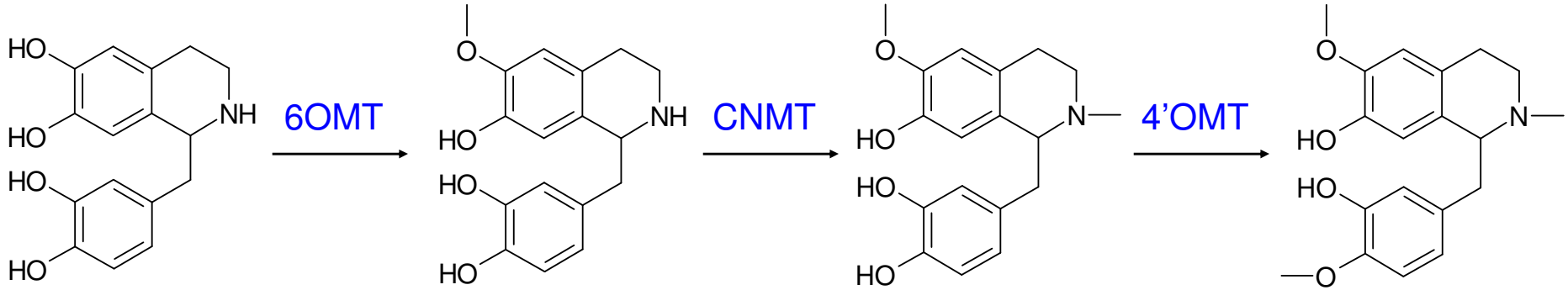
(R,S)-3'-Hydroxy-*N*-methylcoclaurine

(R,S)-Reticuline

Enzyme level dependence:



Optimization of (R,S)-Reticuline production



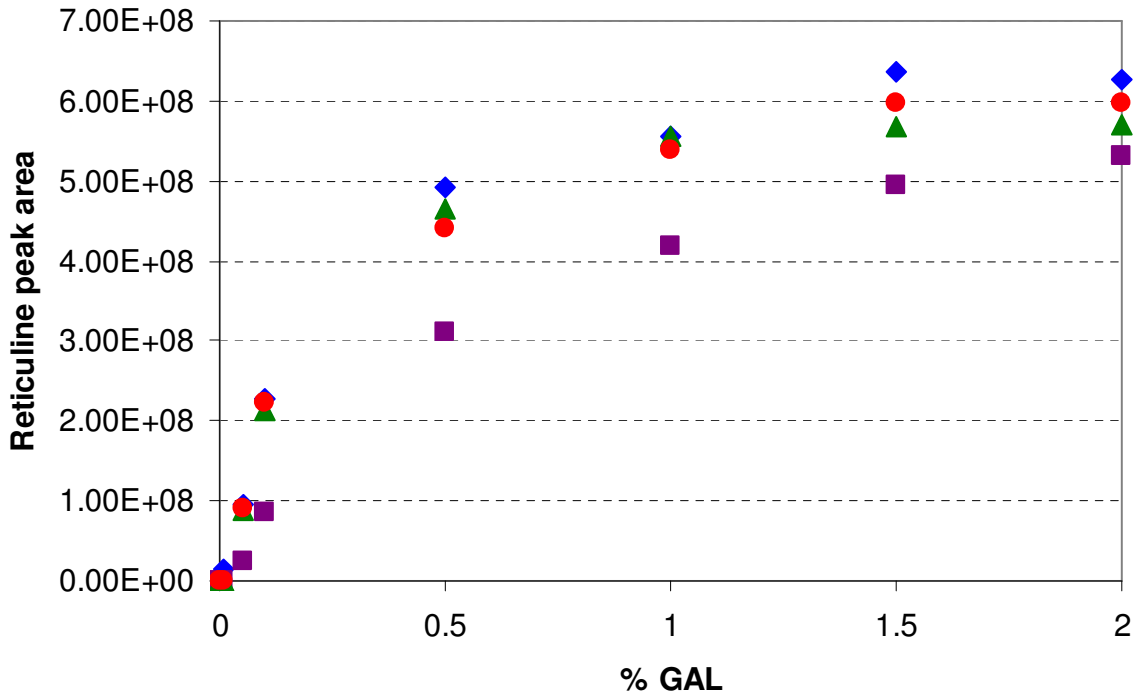
(R,S)-Norlaudanosoline

(R,S)-6-*O*-Methyl-norlaudanosoline

(R,S)-3'-Hydroxy-*N*-methylcoclaurine

(R,S)-Reticuline

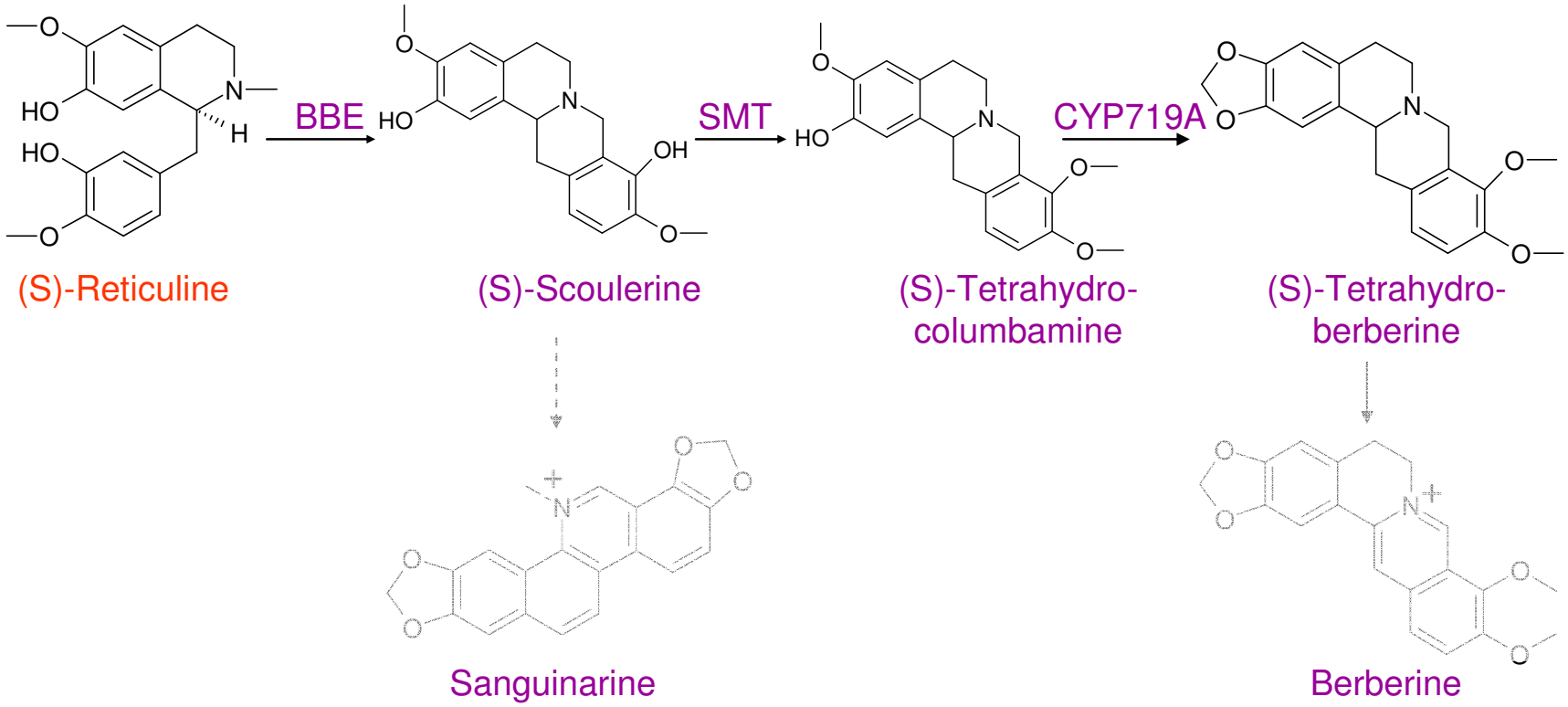
Enzyme level dependence:



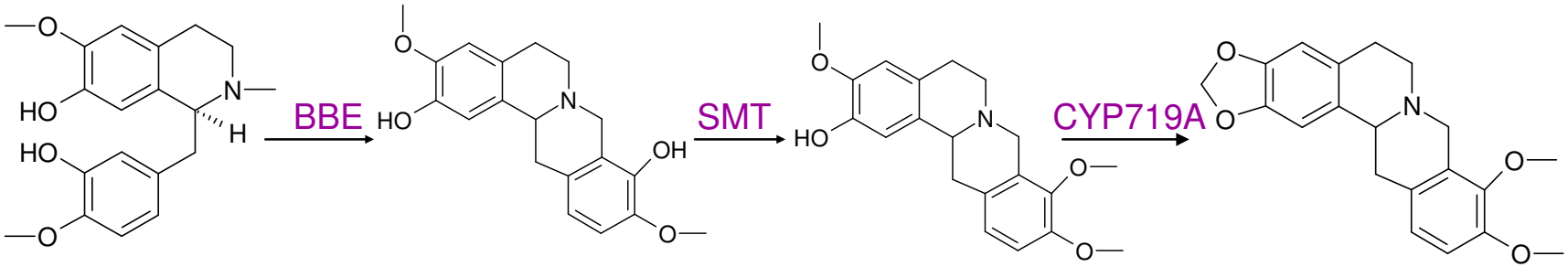
- P_{GAL}Ps6OMT
- P_{GAL}Ps4O'MT
- P_{GAL}PsCNMT
- P_{GAL}PsCNMT P_{TEF}Tf4'OMT

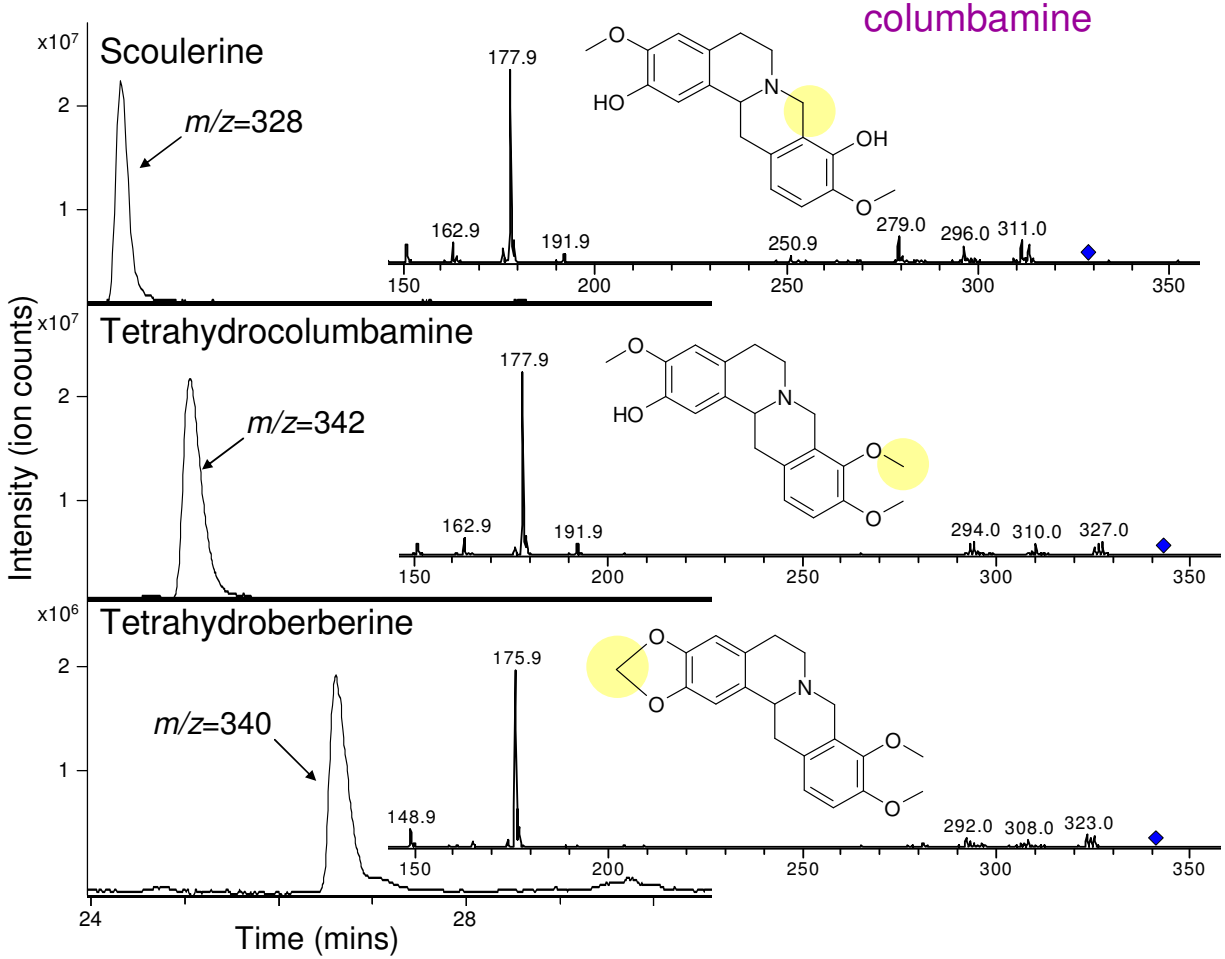
~15% full expression levels from P_{TET} sufficient for maximum conversion

BIA synthesis beyond reticuline – berberine branch



BIA synthesis beyond reticuline – berberine branch



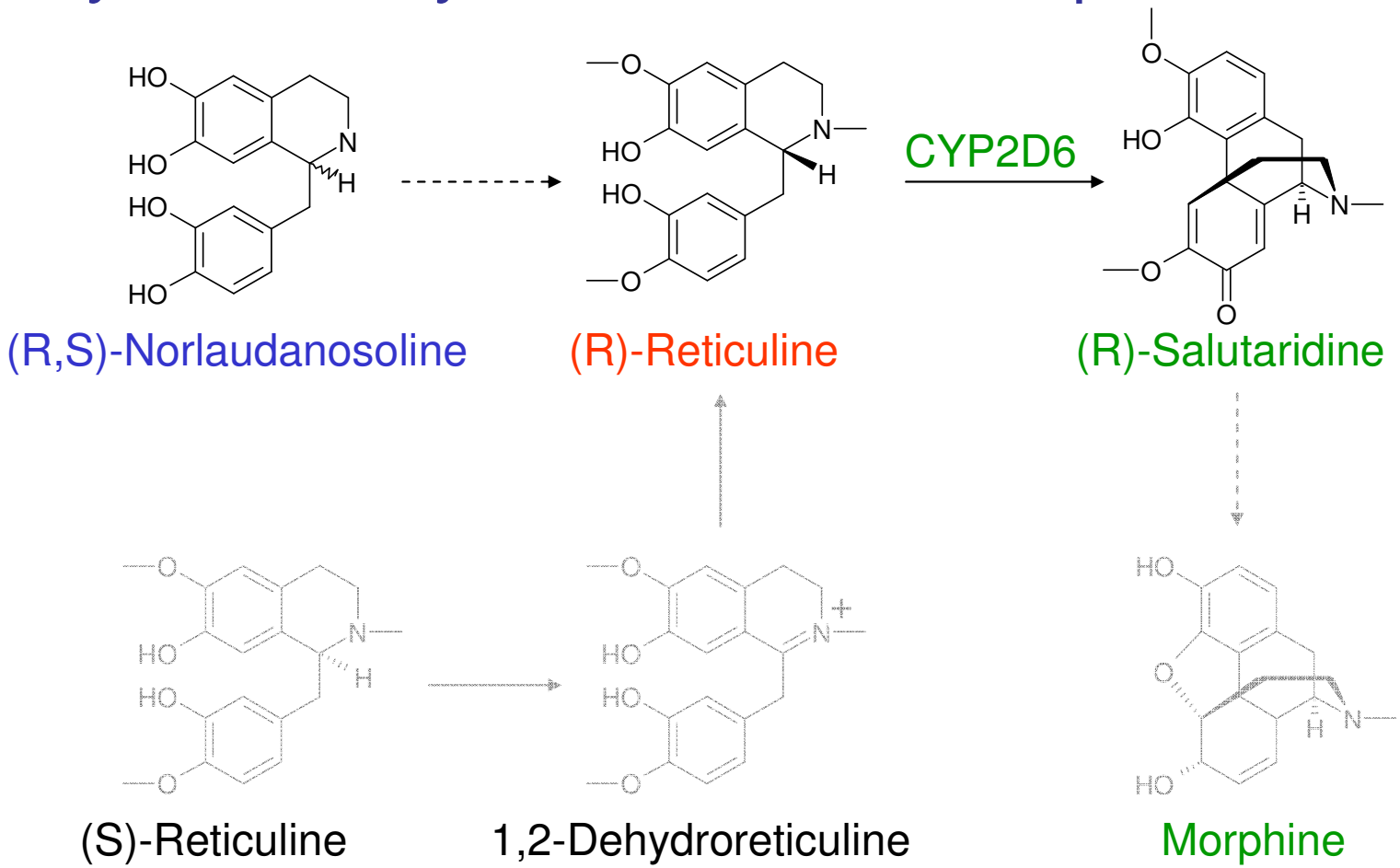


PsBBE

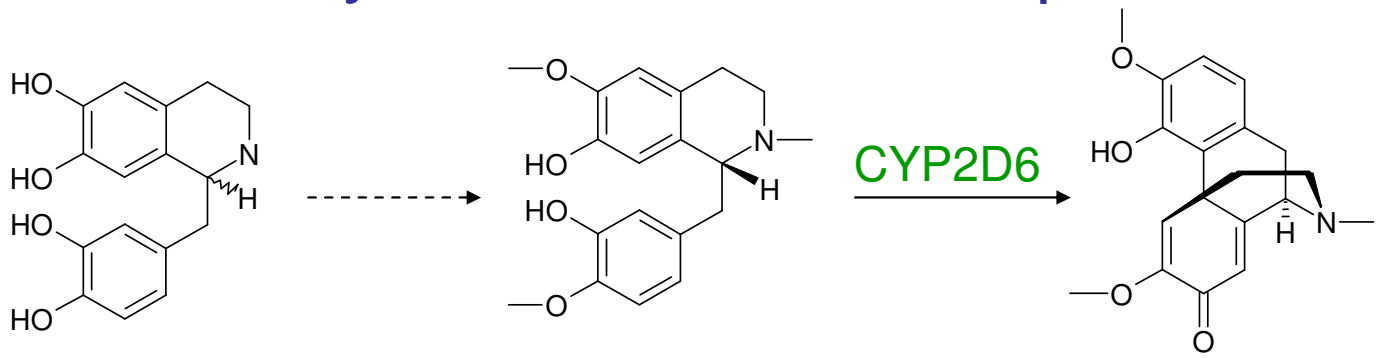
TfSMT

TfCYP719A
AtATR1

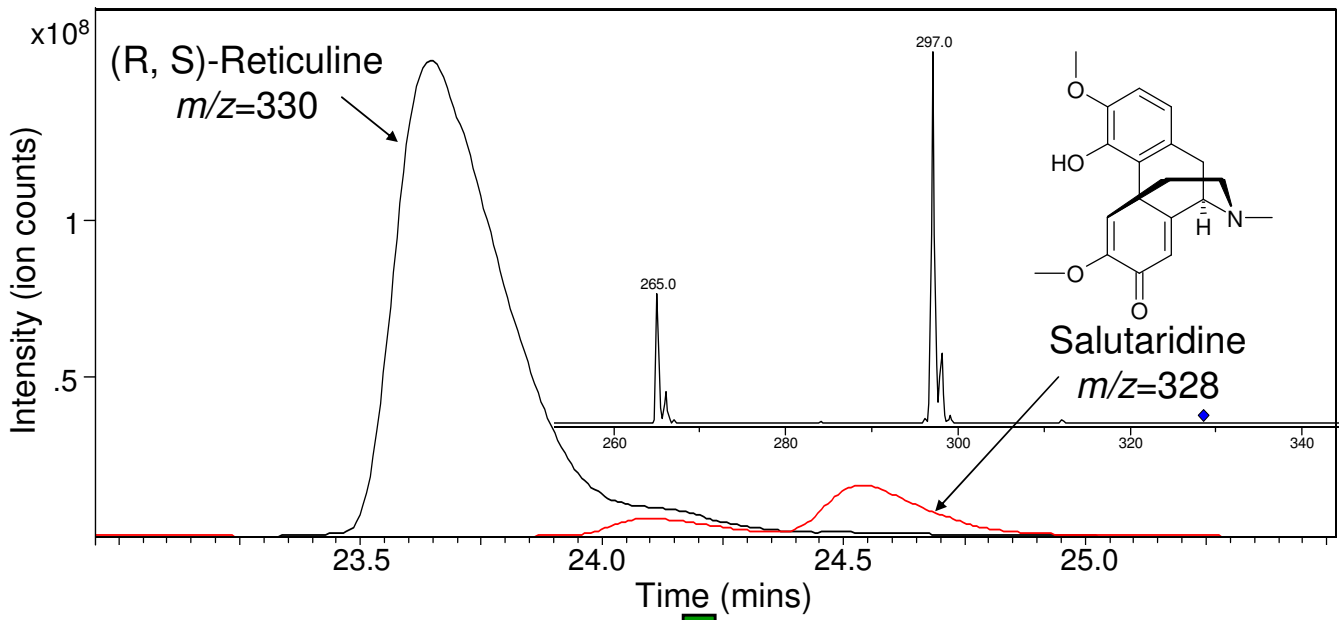
BIA synthesis beyond reticuline – morphine branch



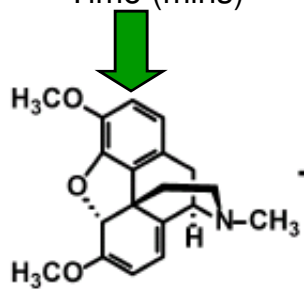
BIA synthesis beyond reticuline – morphine branch



(R,S)-Norlaudanosoline (R)-Reticuline (R)-Salutaridine

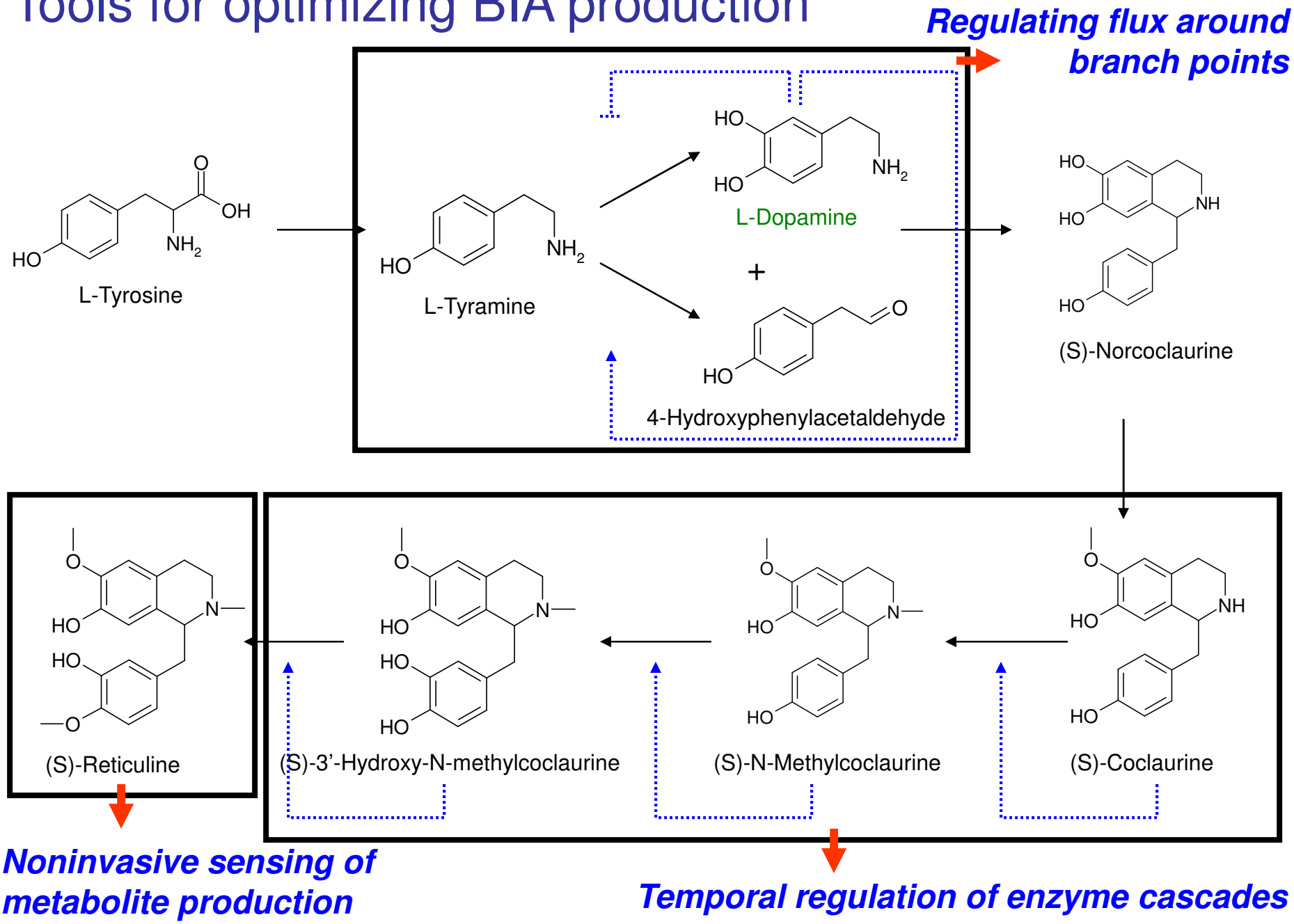


HsCYP2D6

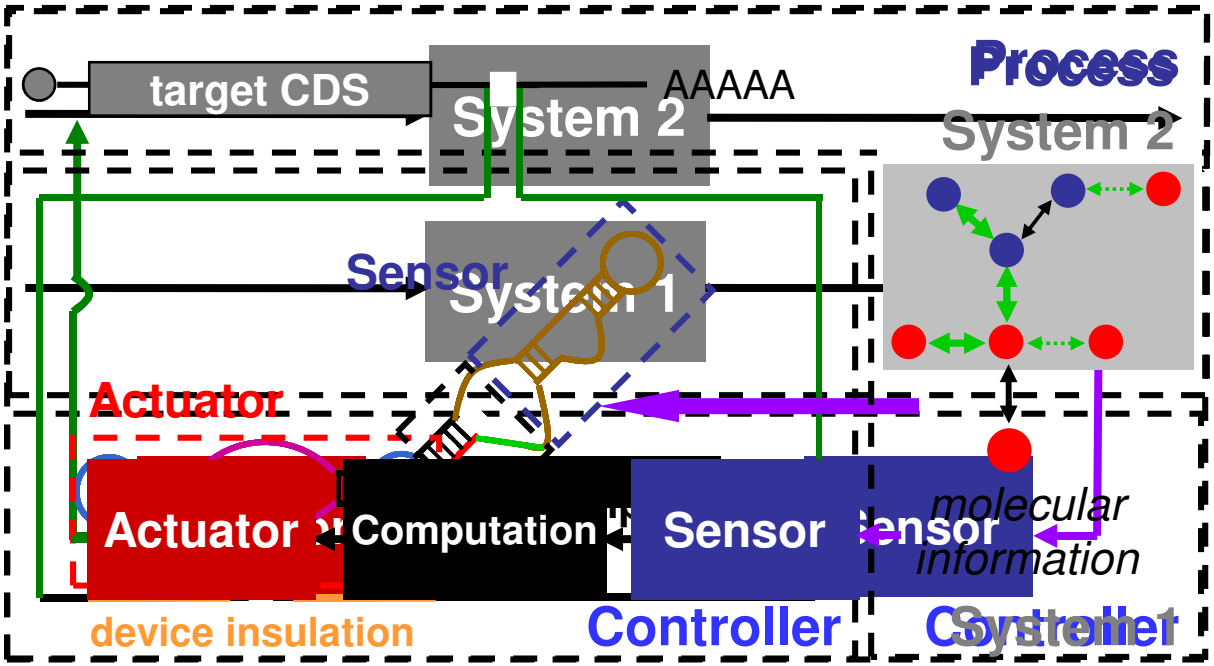


Thebaine

Tools for optimizing BIA production

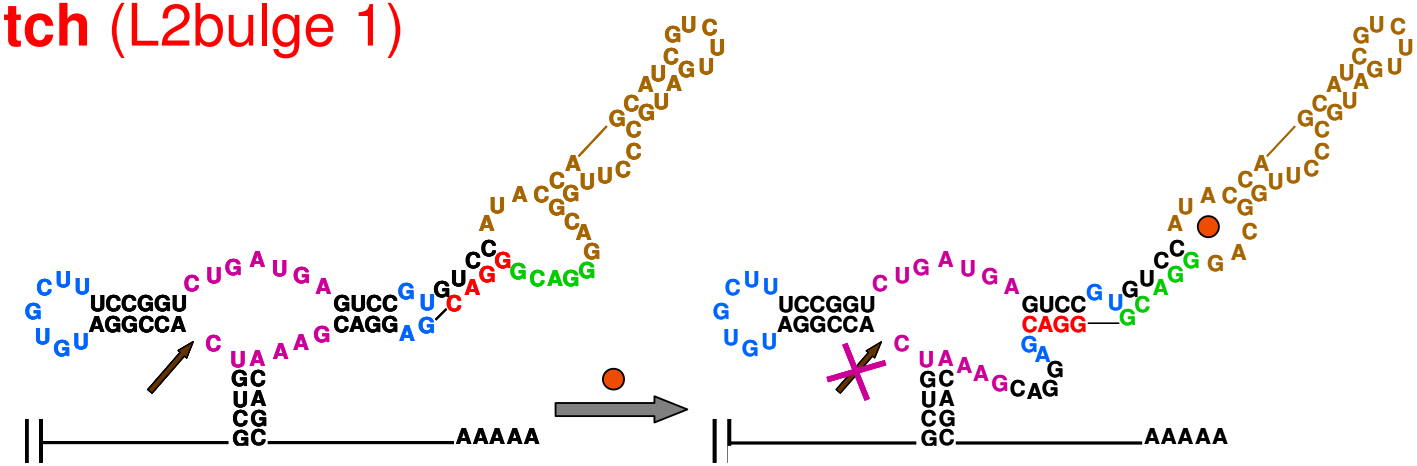


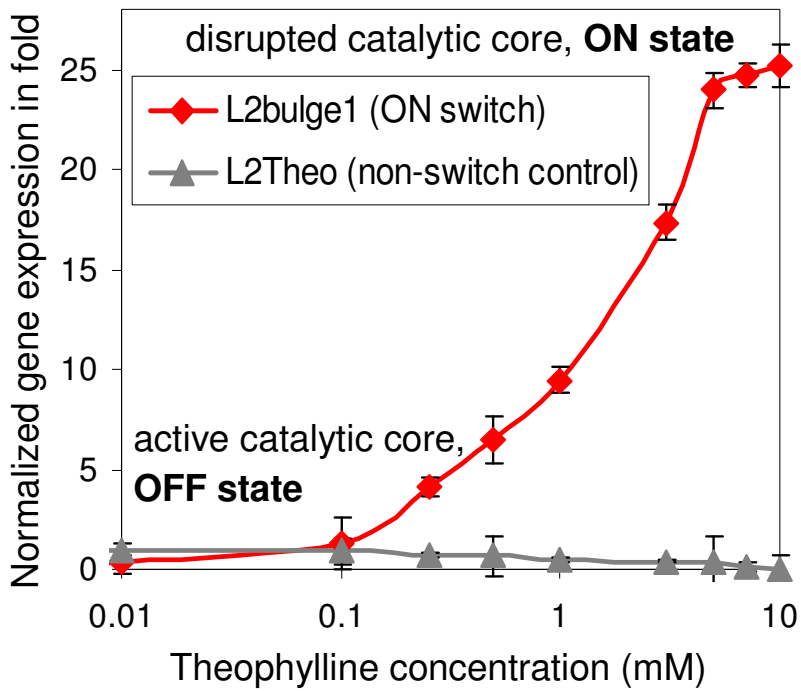
General biological control system



A ribozyme switch platform for up-regulating expression

ON switch (L2bulge 1)

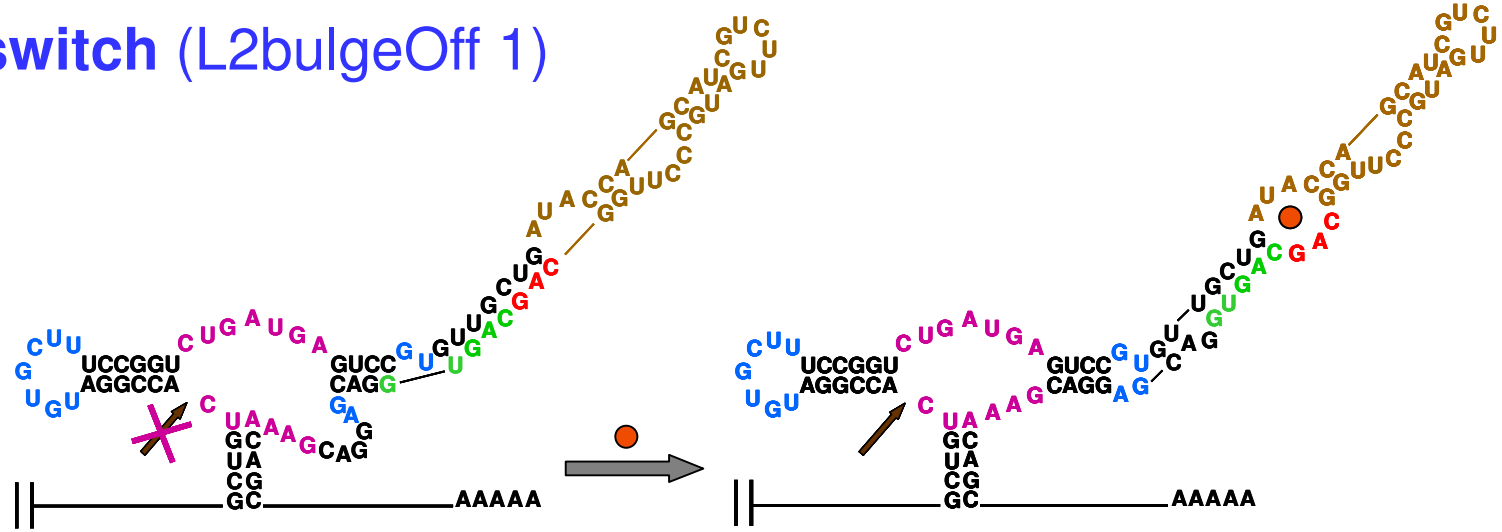


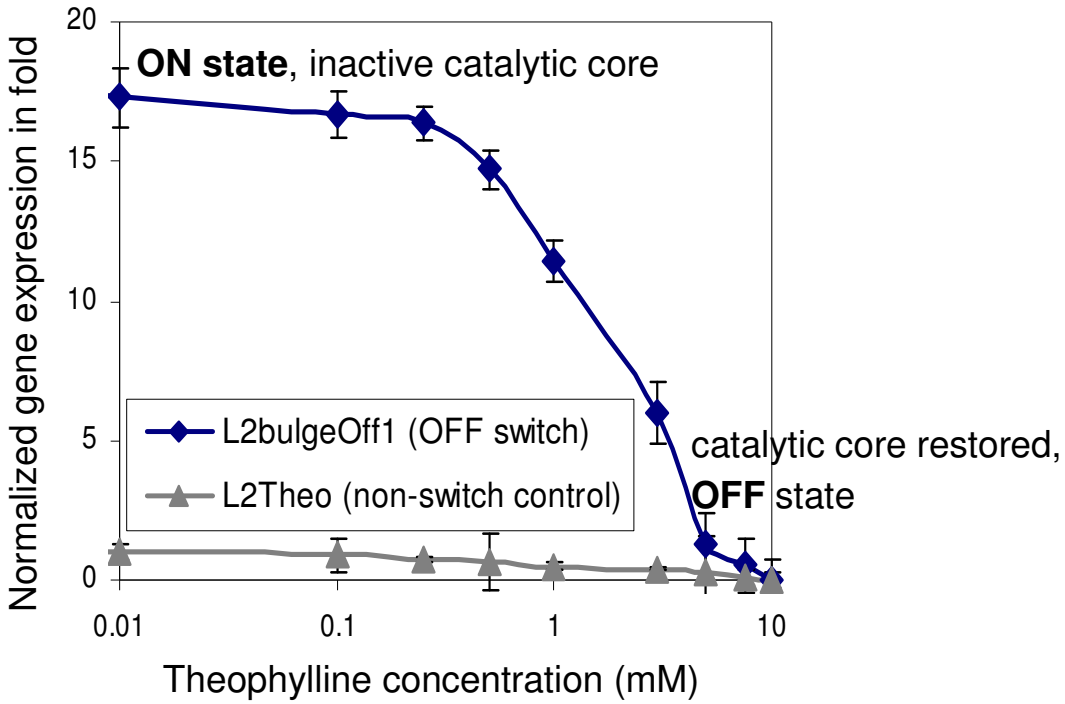


Win MN, Smolke CD. 2007. *PNAS*. 104: 14283-8

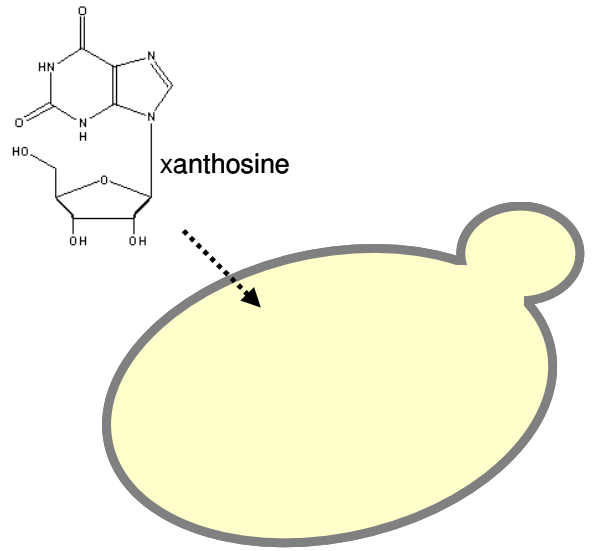
A ribozyme switch platform for down-regulating expression

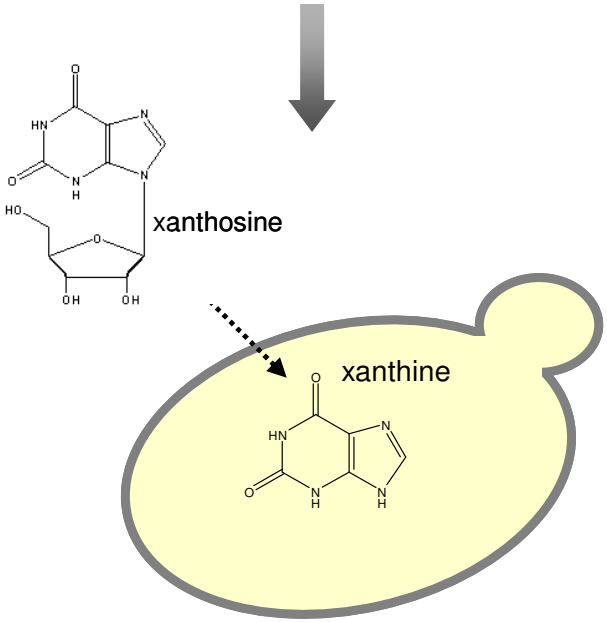
OFF switch (L2bulgeOff 1)



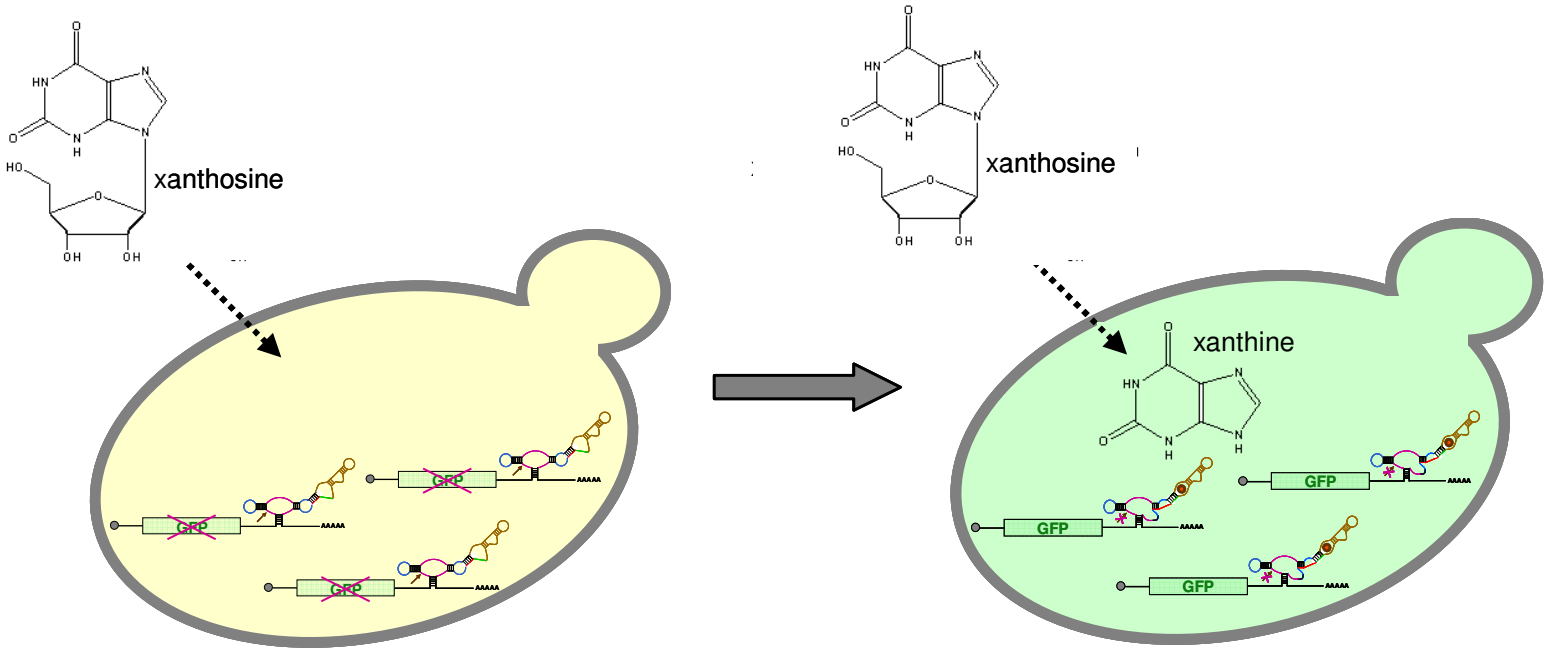


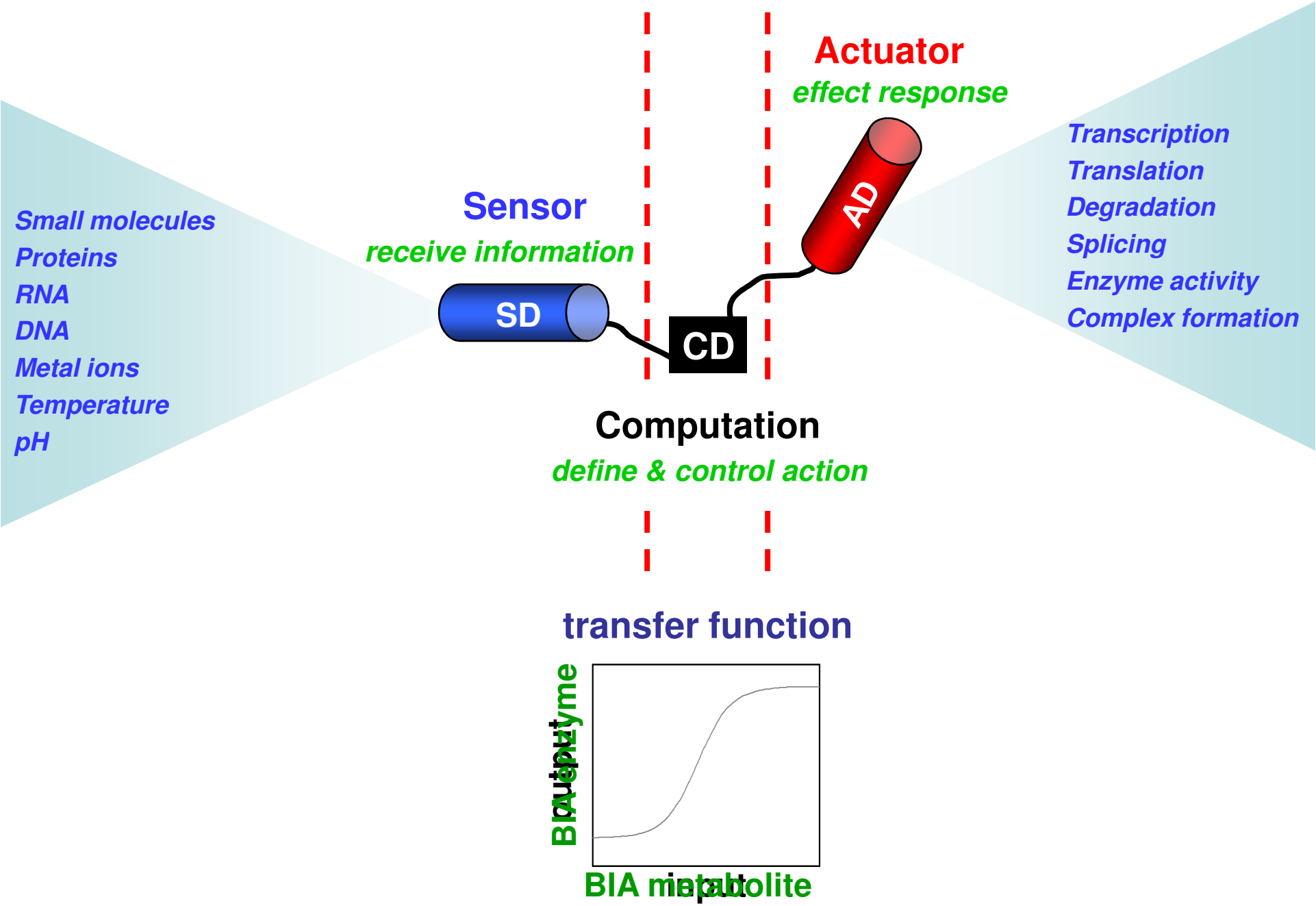
Integrating RNA devices as noninvasive sensors of metabolite concentration





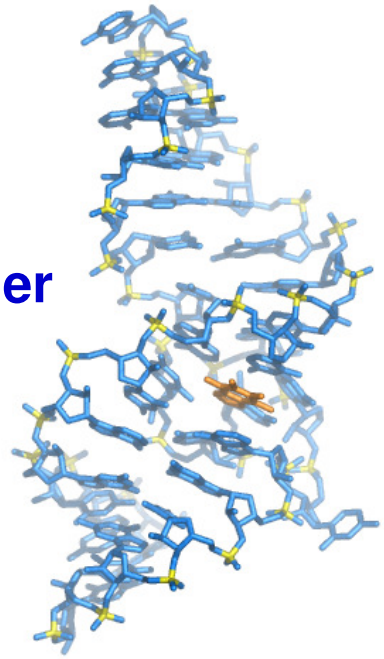
Integrating RNA devices as noninvasive sensors of metabolite concentration



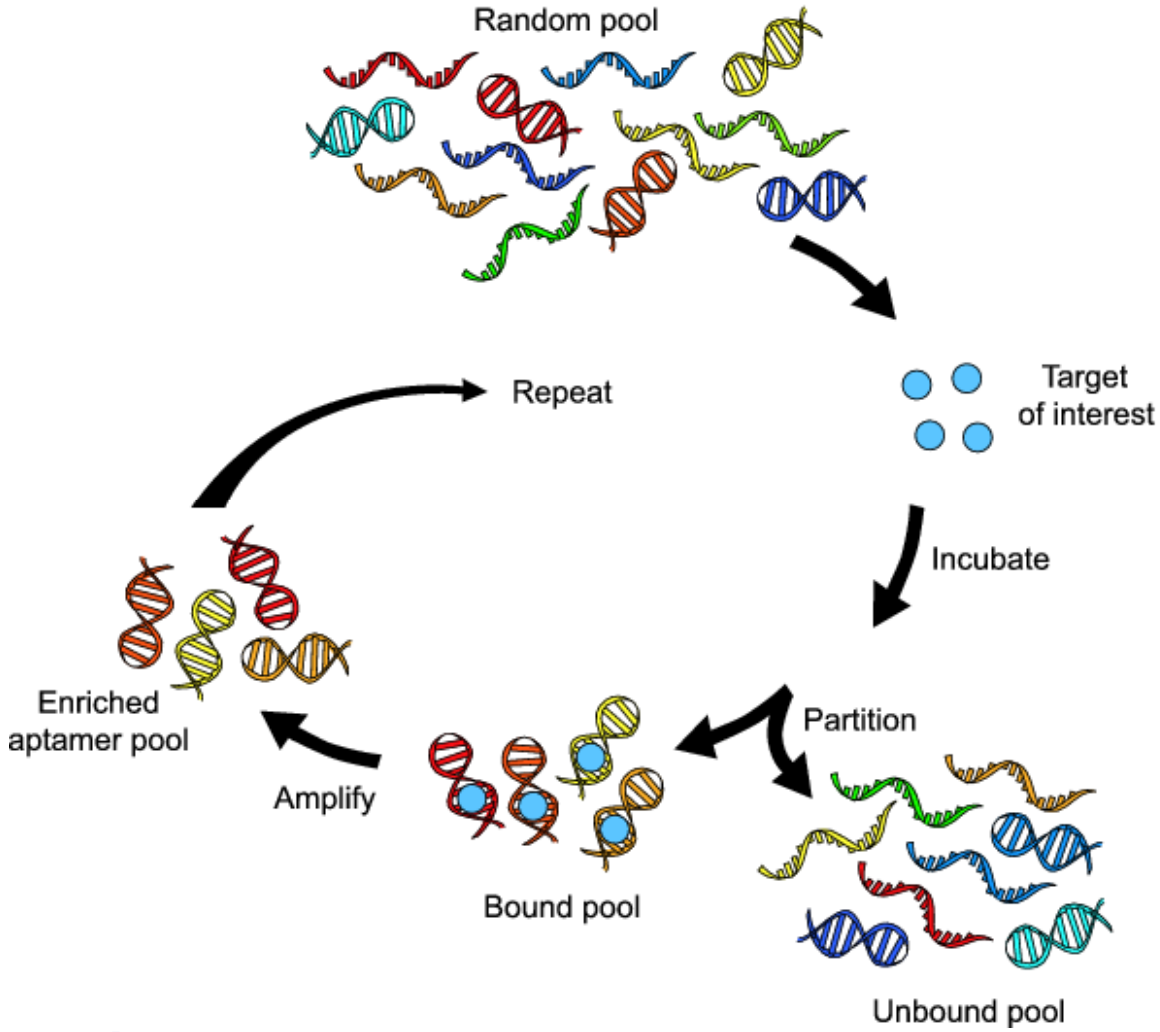

Small molecules
Proteins
RNA
DNA
Metal ions
Temperature
pH
Sensor
receive information
SD
CD
Actuator
effect response
AD
Transcription
Translation
Degradation
Splicing
Enzyme activity
Complex formation
Computation
define & control action
transfer function
BIA output
BIA input

Scalability challenge: libraries of nucleic acid sensors

aptamer

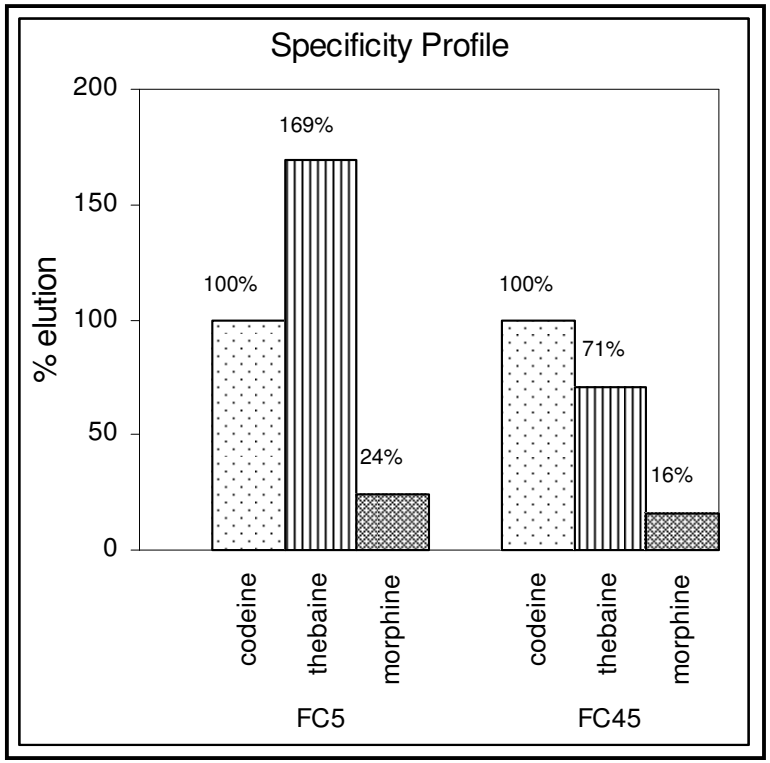


- bind wide range of ligands
- high specificity and affinity
- generated through *in vitro* selection process

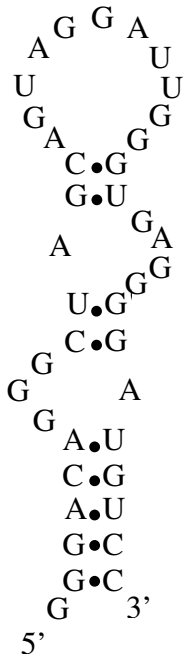


aptamer selection scheme

Specificities of BIA-binding aptamers

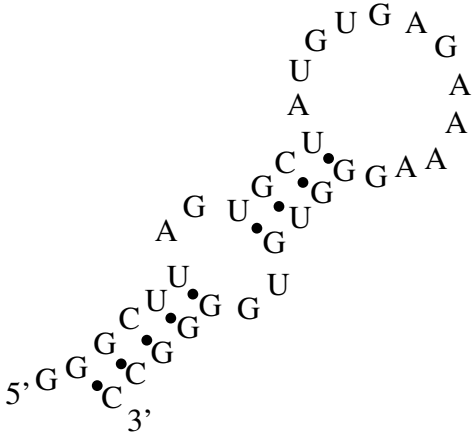


FC5

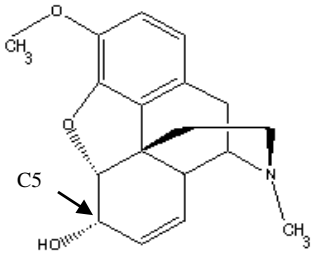


K_d (FC5L) = 4.55±0.14 µM

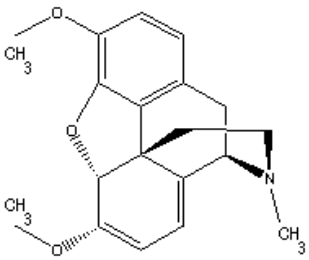
FC45



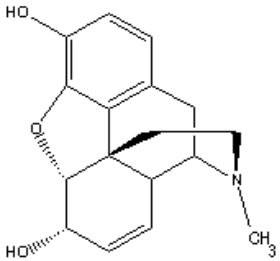
Kd (FC45L) = 2.59 ± 0.09 µM



codeine



thebaine



morphine

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

Win MN, Klein JS, Smolke CD. 2006. *Nuc Acids Res.* 34: 5670-82.

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

Kevin Hoff

Graduate Researchers

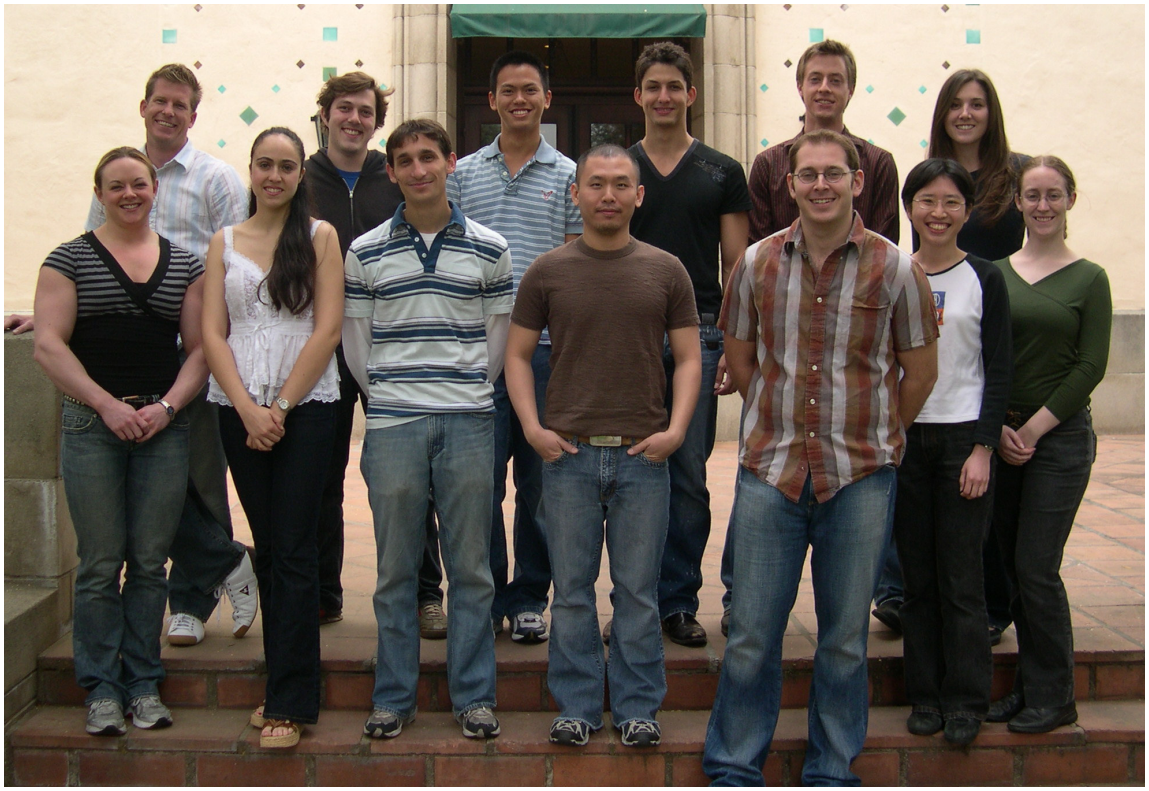
Andrew Babiskin
Chase Beisel
Arwen Brown
Yvonne Chen
Stephanie Culler
Leo d'Espaux
Katie Galloway
Kristy Hawkins
Joe Liang
Josh Michener
Michael Siddiqui
Jay Vowles
Maung Nyan Win

Technicians

Midori Greenwood-Goodwin

Collaborators

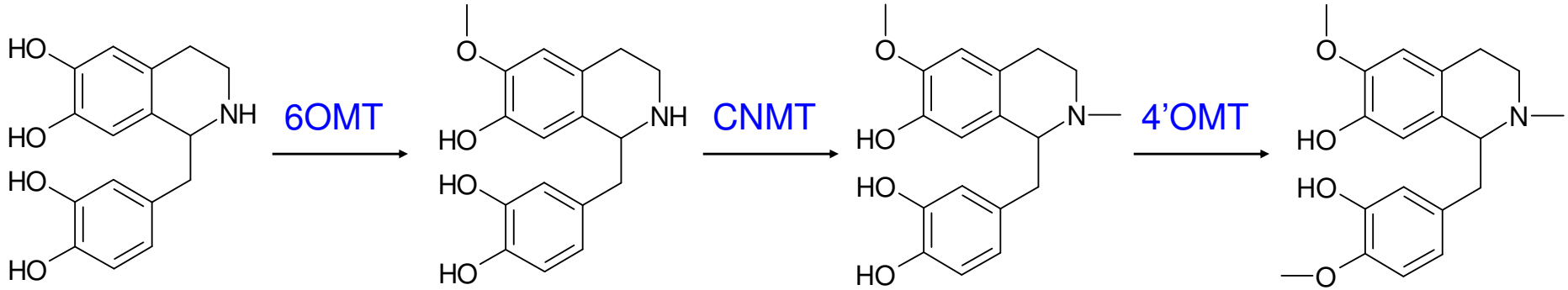
The Facchini Lab (U. Calgary)
The Jensen Lab (COH)



Funding Sources

Arnold and Mabel Beckman Foundation
Caltech
Center for Biological Circuit Design (Caltech)
City of Hope Cancer Center
Defense Advanced Research Projects Agency
Department of Defense (BCRP)
Grubstake Program (OTT Caltech)
Joseph Jacobs Institute for Molecular Engineering for Medicine (Caltech)
National Institutes of Health (NCI)
National Institutes of Health (NIGMS)
National Science Foundation (BES)

Optimization of (R,S)-Reticuline production



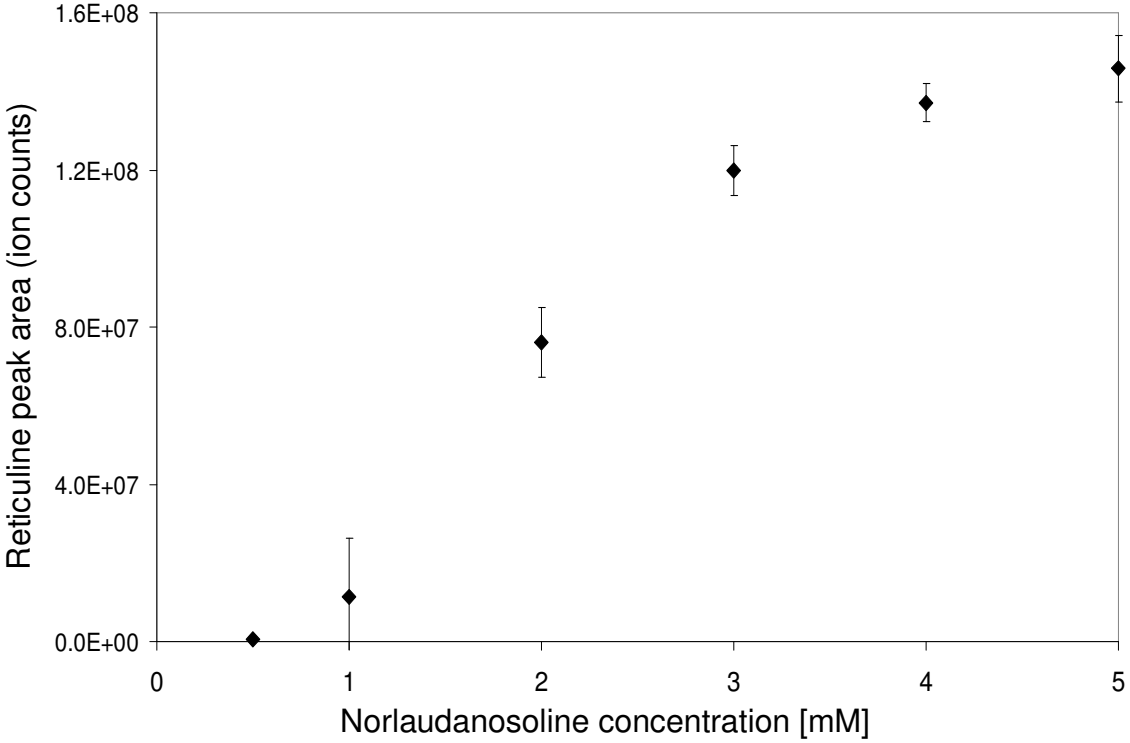
(R,S)-Norlaudanosoline

(R,S)-6-*O*-Methyl-norlaudanosoline

(R,S)-3'-Hydroxy-*N*-methylcoclaurine

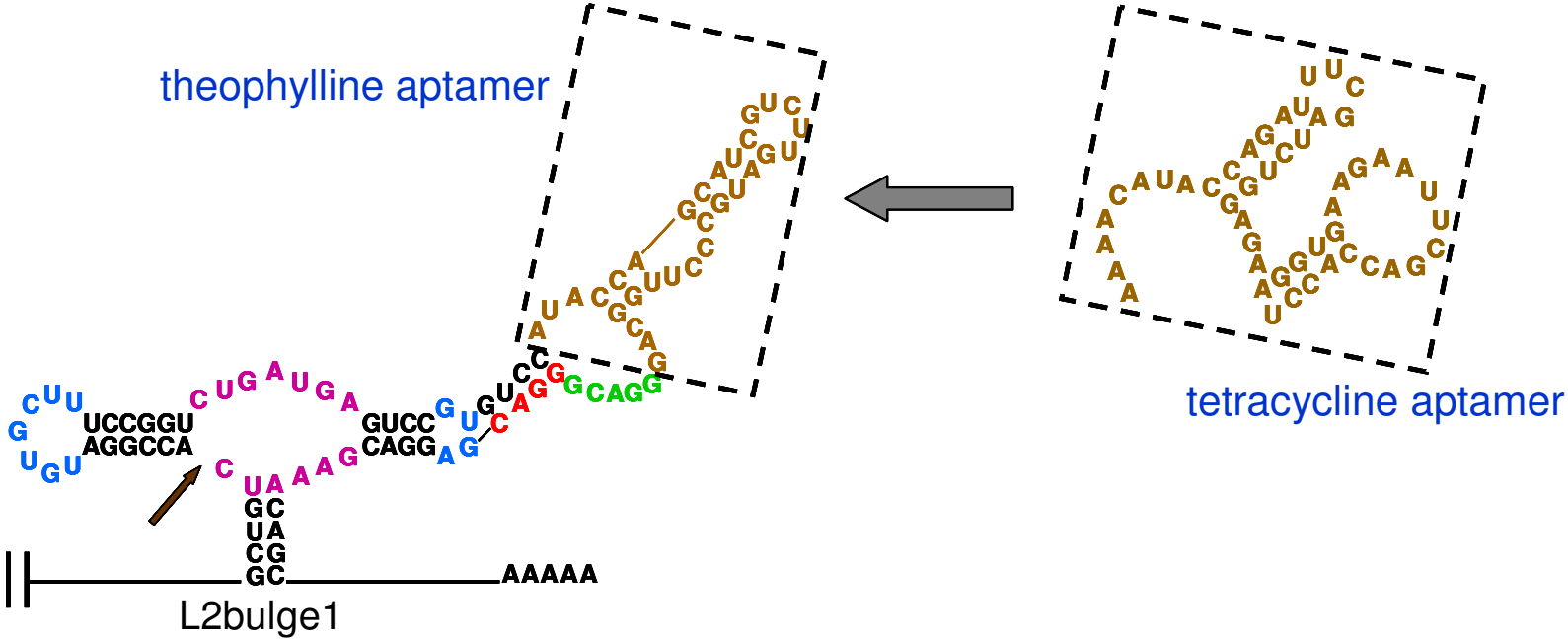
(R,S)-Reticuline

Substrate level dependence:

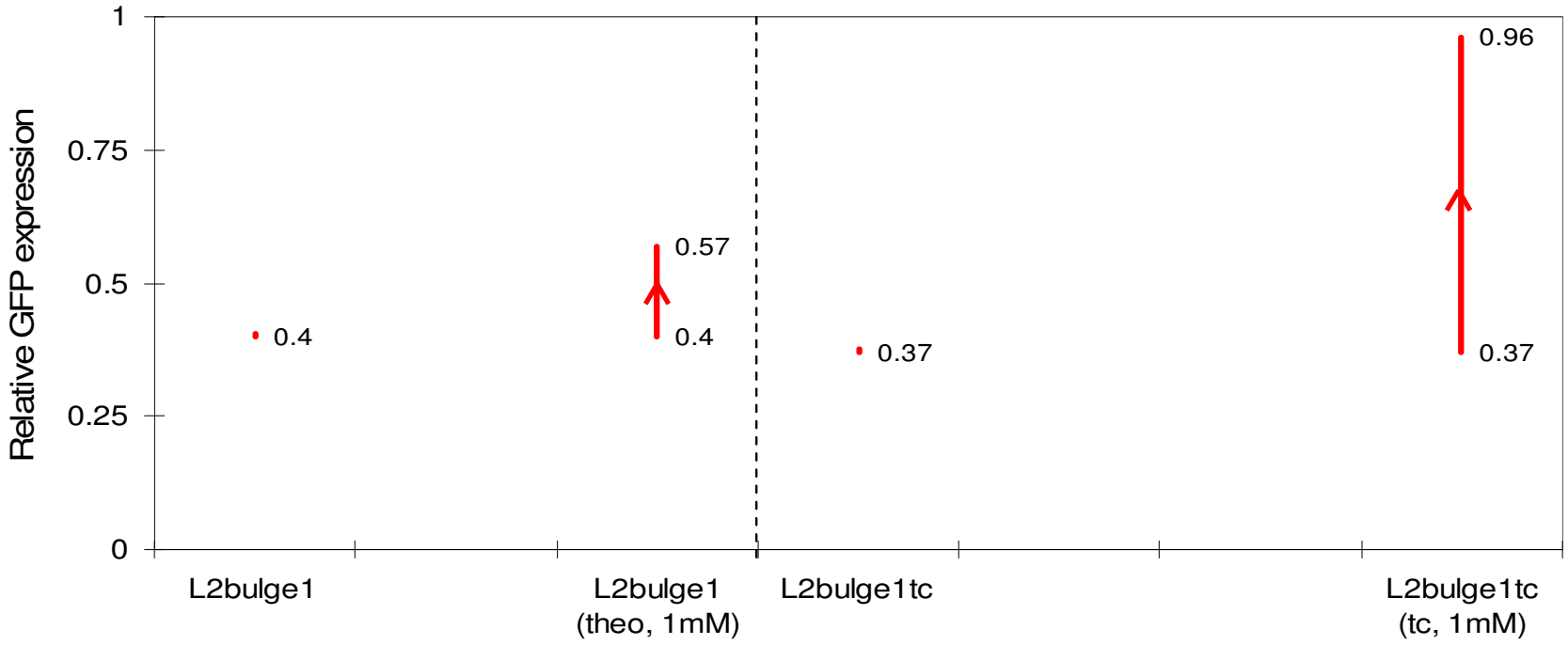


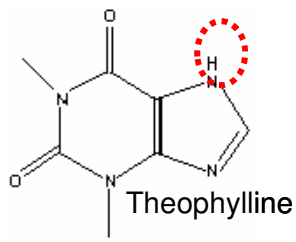
Substrate concentration is limiting and not enzyme levels.

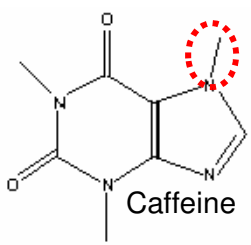
Modularity of the sensor domain

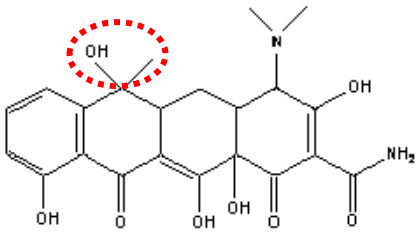


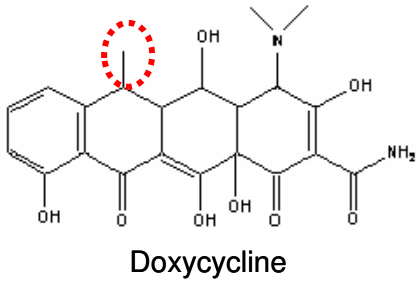
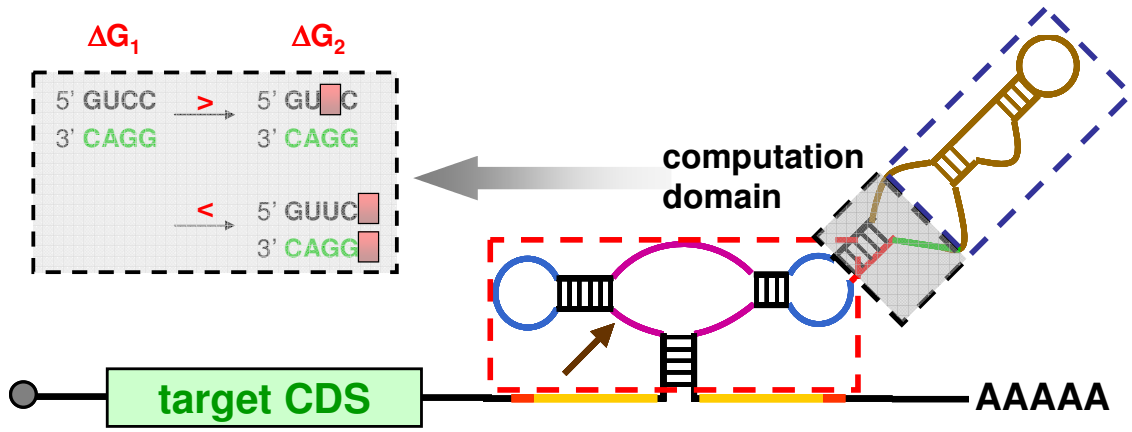
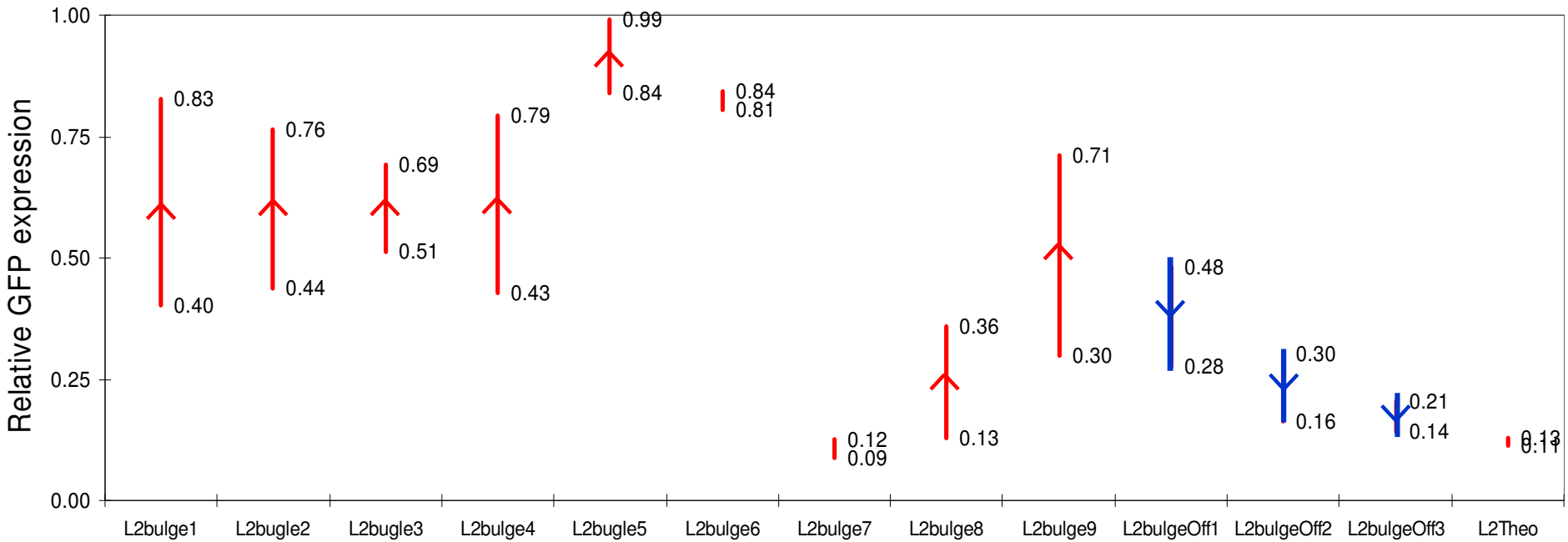
Modularity of the sensor domain











Programming response properties of ribozyme switches