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# 2014 REU Poster: Measuring Tryptophan Metabolism Using Analogues of Tryptophan

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# Measuring Tryptophan Metabolism Using Analogues of Tryptophan



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## Introduction

- Secondary metabolites are organic compounds produced through the modification of primary metabolites. Secondary metabolites typically do not play essential roles in growth as primary metabolites do.
- In plant, most secondary metabolites play roles in defense against insects and microbial pathogens.
- Many plant secondary chemicals have important uses for humans. For example, many pharmaceuticals are based on plant chemical structures, and secondary metabolites are widely used for recreation and stimulation.
- Arabidopsis thaliana* is a small flowering plant that is widely used as a model organism in plant biology. *Arabidopsis* is a member of the mustard (Brassicaceae) family, which includes cultivated species such as cabbage and radish.

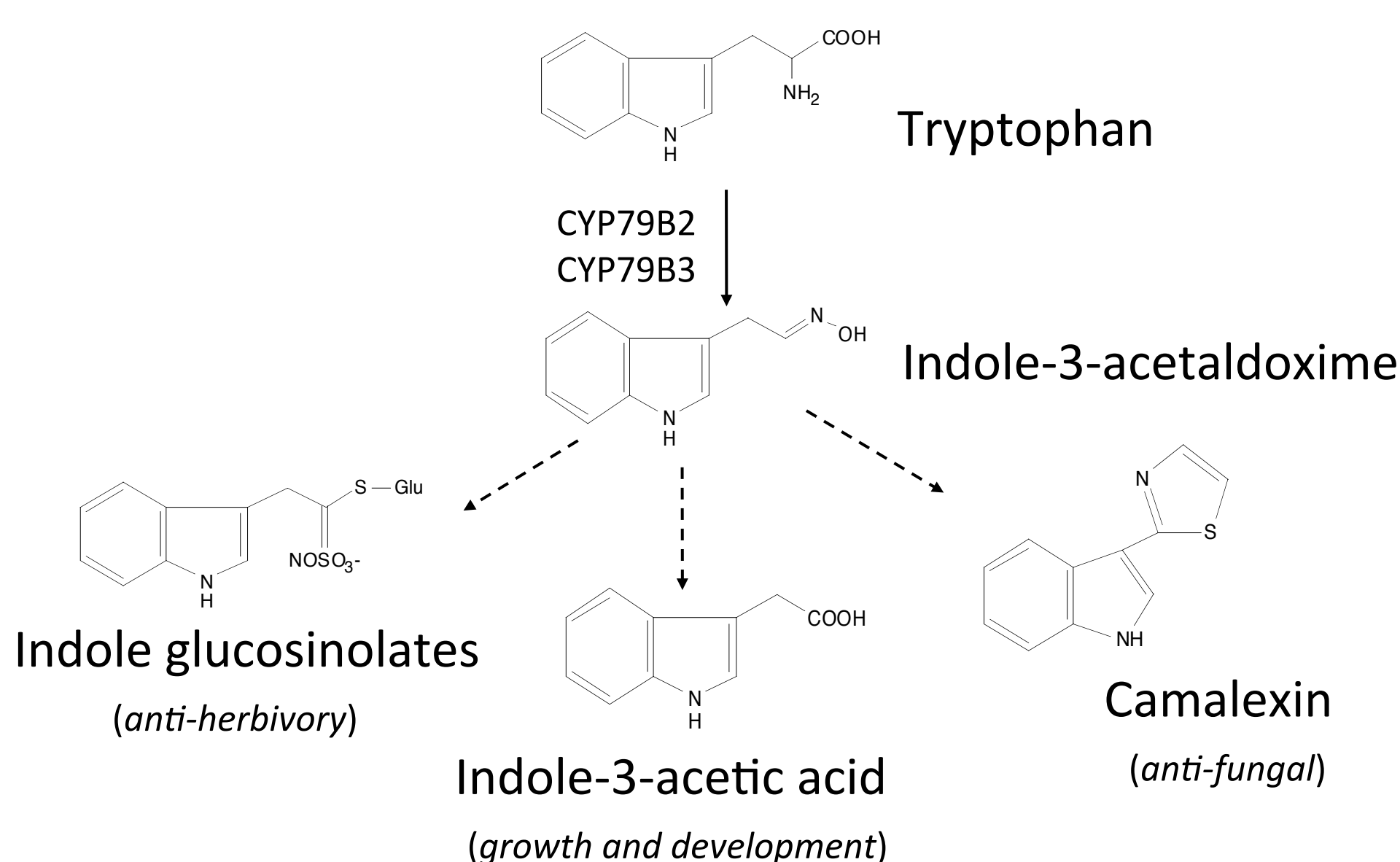
## Why we use Arabidopsis



- Cheap/easy to maintain
- Self fertile
- Fast life cycle
- Transformable
- 5 chromosomes (diploid)
- Small genome: approx. 26,000

## In Arabidopsis, tryptophan is the precursor of several secondary metabolites

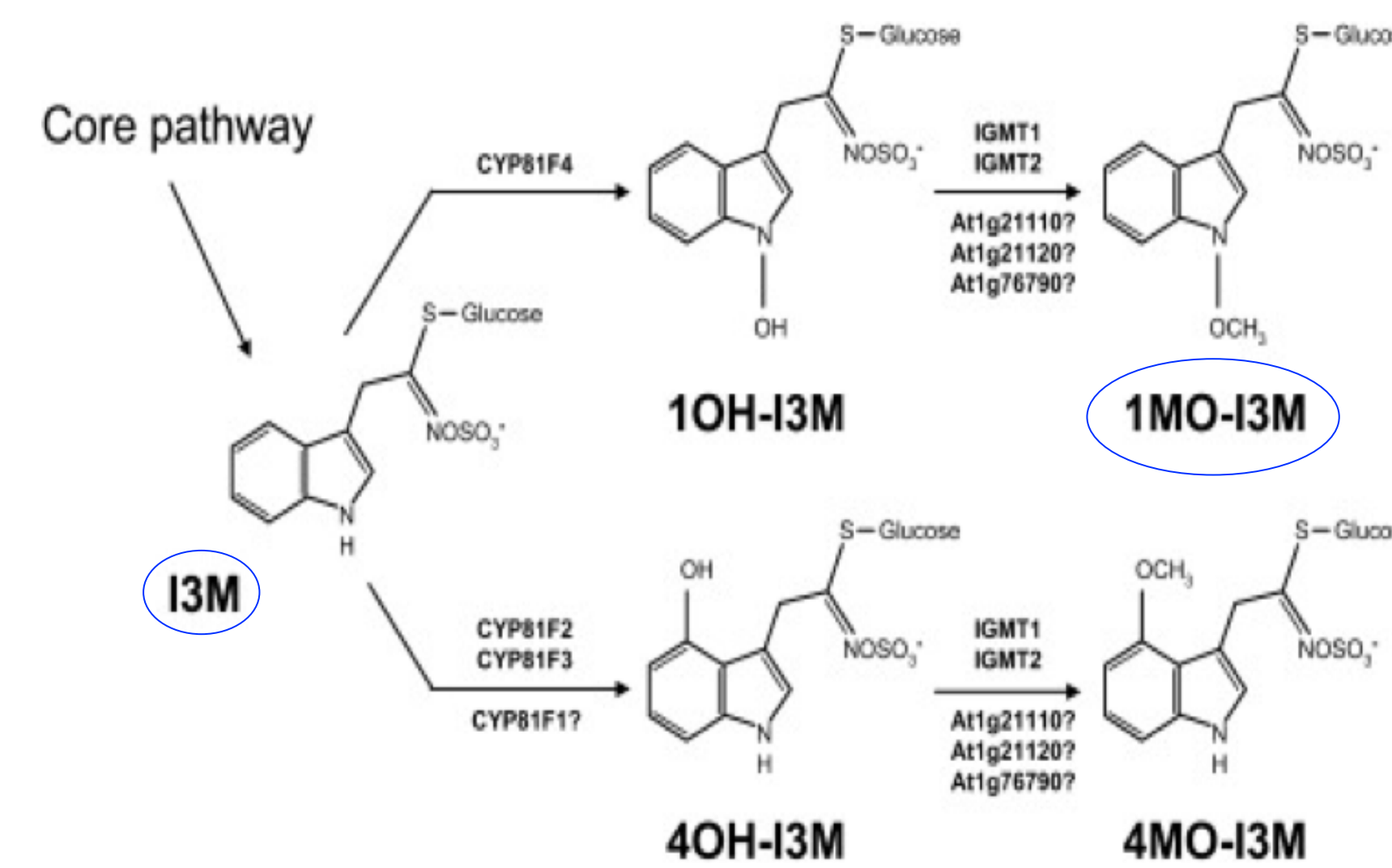
- Glucosinolates are a class of defense compound used to protect the plant from insect attack. Those derived from tryptophan (Trp) are called indolic glucosinolates (IGs).
- Camalexin (CAM) is another defense compound produced from Trp and it protects the plant from fungal attack.
- Indole-3-acetic acid (IAA) is a plant growth hormone called auxin.



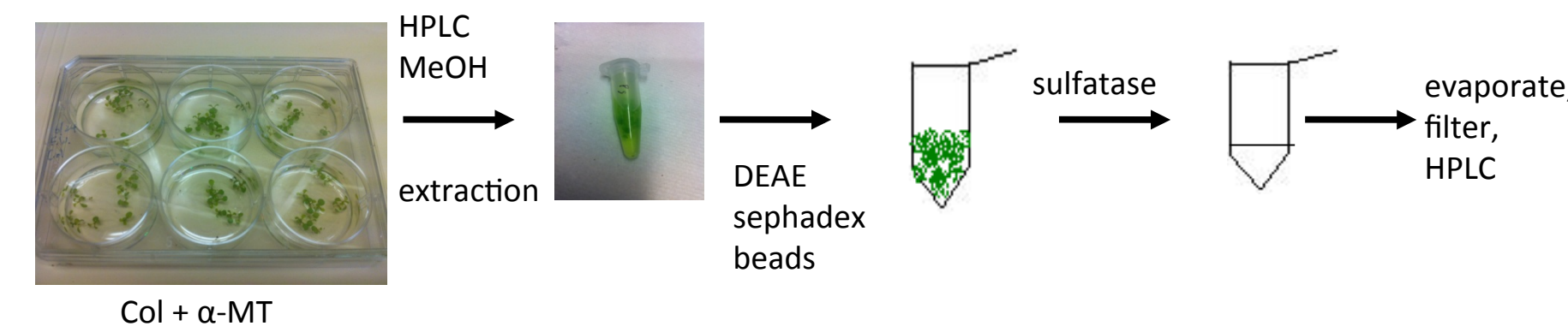
The enzymes CYP79B2 and CYP79B3 cleave off the Trp carboxyl group and then hydroxylate to form indole-3-acetaldoxime from which are derived IGs, IAA and CAM.

## Indolic glucosinolates made by Arabidopsis

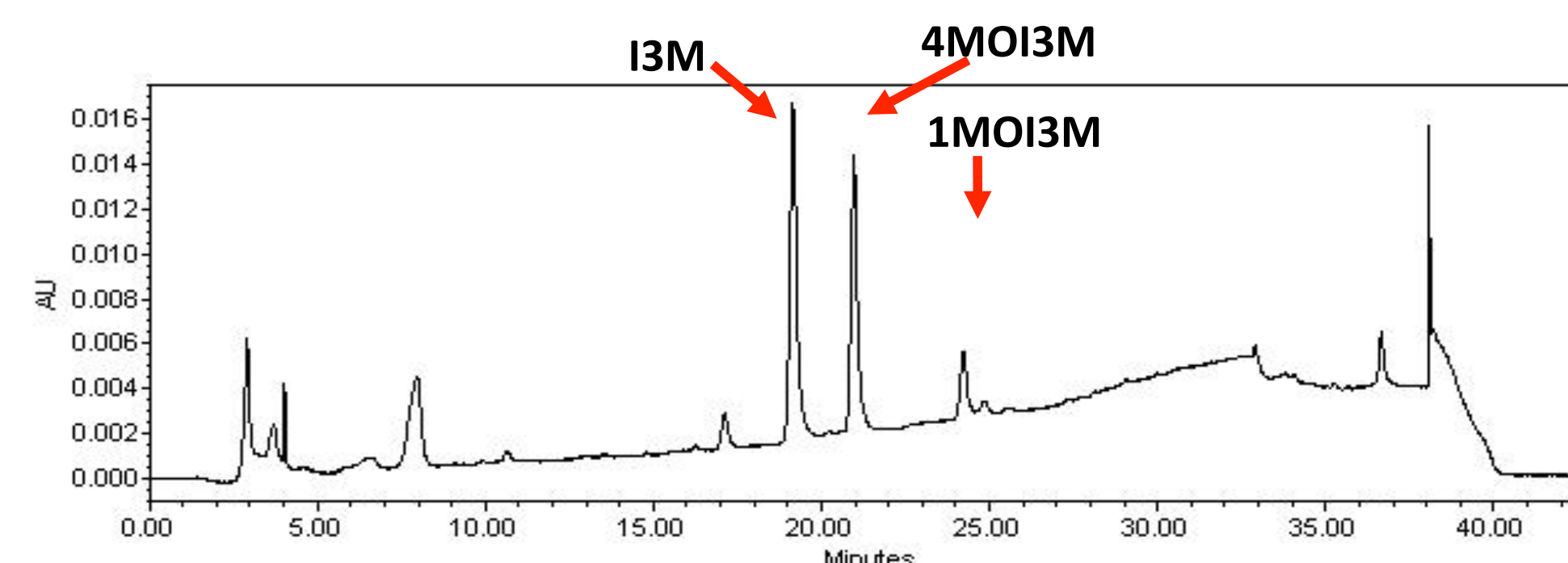
(circled compounds accumulate to measurable quantities)



## IG Quantification



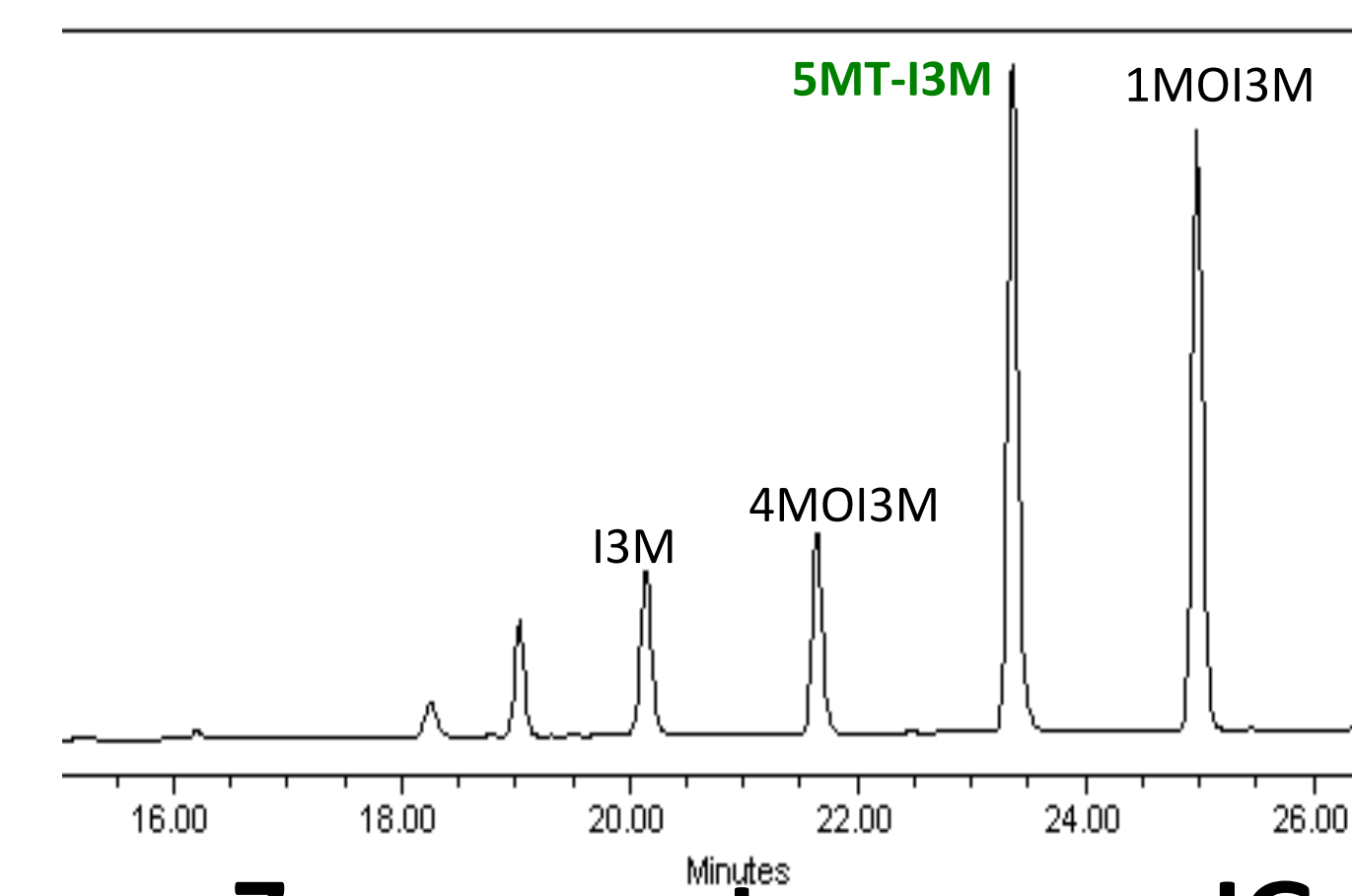
- Plants are grown in sterile liquid PNS media and at 10 days after germination they are fed with 25 micromolar 7AT or 5MA and grown for 48 h.
- Plant tissue is harvested and “desulfo-glucosinolates” are isolated
- Desulfoglucosinolates are then analyzed by reverse-phase HPLC using an increasing acetonitrile gradient
- Different desulfoglucosinolates were detected by their absorbance at 229nm.
- For quantification, peak areas were normalized to plant tissue weight and a known weight standard



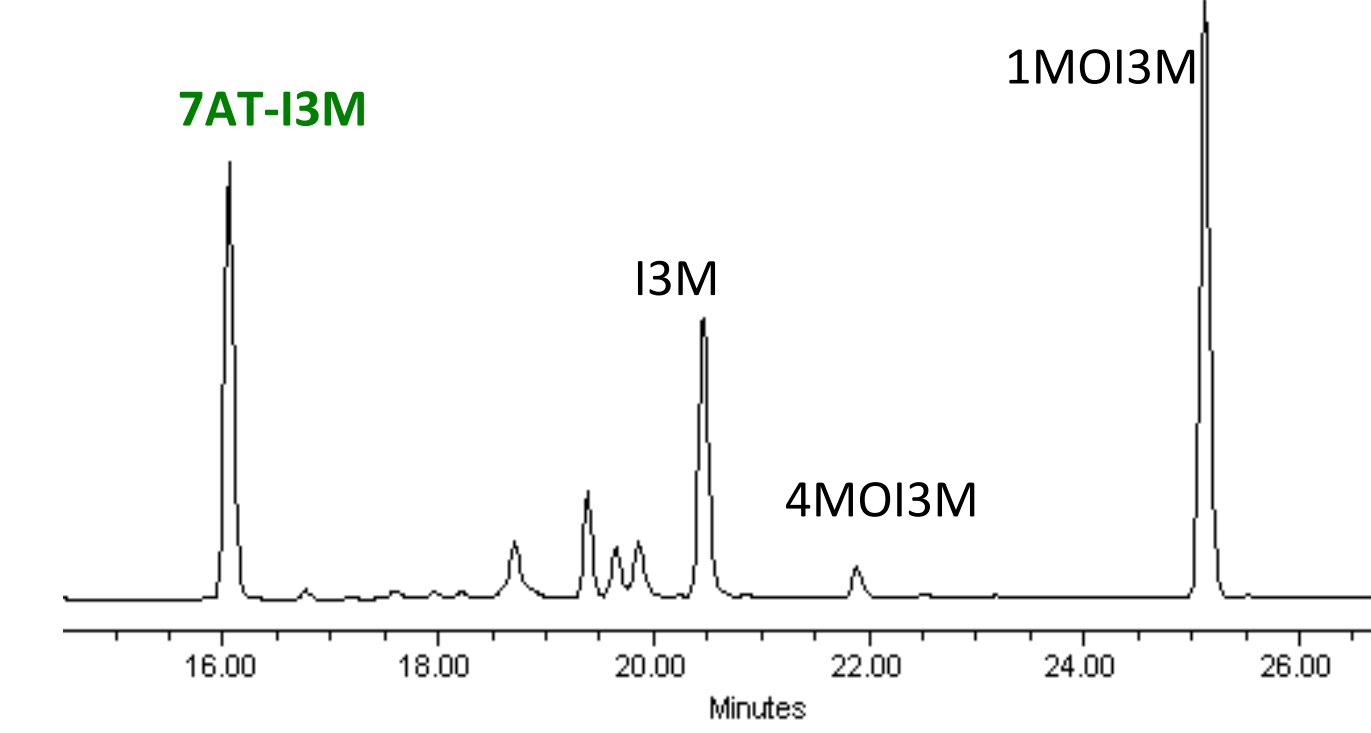
## Hypotheses

- Our first hypothesis is that analogs of tryptophan (Trp) can be converted into novel indolic glucosinolates (IGs) in vivo.
- Our second hypothesis is that the ratio of the incorporation of endogenous Trp into IGs compared to Trp analog incorporation into IGs will depend on the size of the endogenous Trp pool.

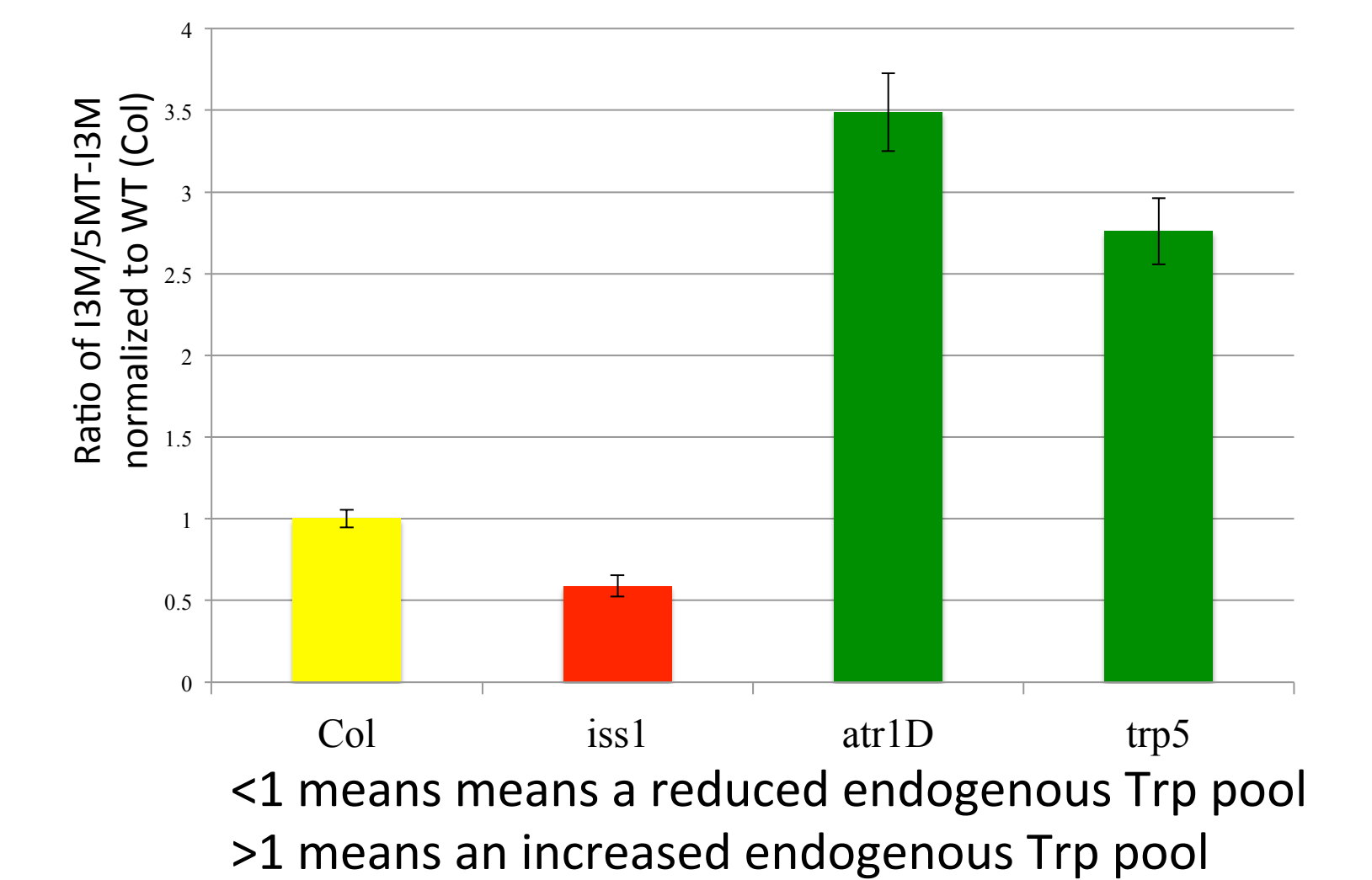
## Hypothesis 1 5-methyl-tryptophan (or 5-methylanthranilate) creates a new IG



## 7-aza-creates a new IG



## The ratio of I3M/5MT-I3M is consistent with mutant phenotypes



## Hypothesis 2

### Mutants with altered endogenous Trp pools to be tested for incorporation of Trp analogs

*iss1* – the *iss1* mutant has reduced catabolism of Trp and is predicted to have a smaller Trp pool. This is because the decrease in Trp catabolism will result in feedback inhibition of anthranilate synthase, the enzyme that catalyzes the first committed step in Trp biosynthesis. Trp production, and therefore the Trp pool, will be reduced.

*trp5* – the *trp5* mutant has a larger Trp pool because it carries a mutation in anthranilate synthase that makes the enzyme resistant to feedback inhibition.

*atr1D* – the *atr1D* mutant has increased expression of IG biosynthetic genes. This increased gene expression causes a large increase in IG production and greater demand for Trp. The result is an increased Trp pool.

## Conclusions

- Analogues of Trp (or precursors) can be incorporated into indolic glucosinolate in vivo.
- The incorporation of the analogs into indole glucosinolate relative to the incorporation of endogenous Trp, reflects the size of the endogenous Trp pool.

## References

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