

## Root excretion and accumulation of riboflavin derivatives in iron-deficient Medicago truncatula

Jorge Rodríguez-Celma<sup>\*</sup>, Ana Álvarez-Fernández<sup>\*</sup>, Jesús Orduna<sup>\*\*</sup>,  
Anunciación Abadía<sup>\*</sup>, Javier Abadía<sup>\*</sup>, Ana Flor López-Millán<sup>\*</sup>

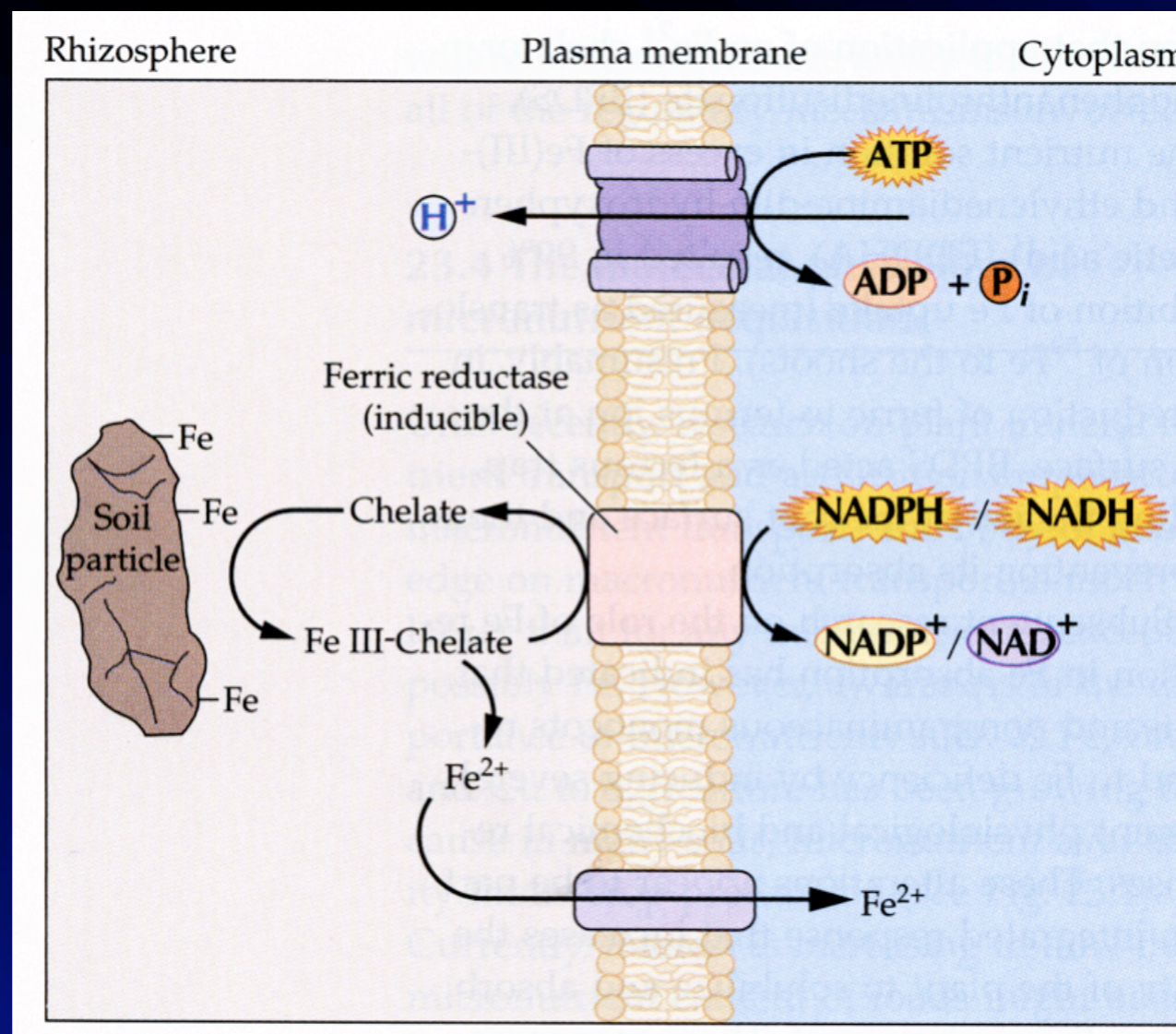
<sup>\*</sup>Departamento de Nutrición Vegetal, Estación Experimental de Aula Dei de Zaragoza, Consejo Superior de Investigaciones Científicas (EEAD-CSIC), Spain; <sup>\*\*</sup>New Organic Materials Department, Institute of Materials Sciences, Consejo Superior de Investigaciones Científicas-Universidad de Zaragoza (ICMA-CSIC), Spain



## Strategy I: Morphological and biochemical changes

phenolic compounds  
flavins

organic acids  
flavins



Buchanan, Grissem and Jones, 2000

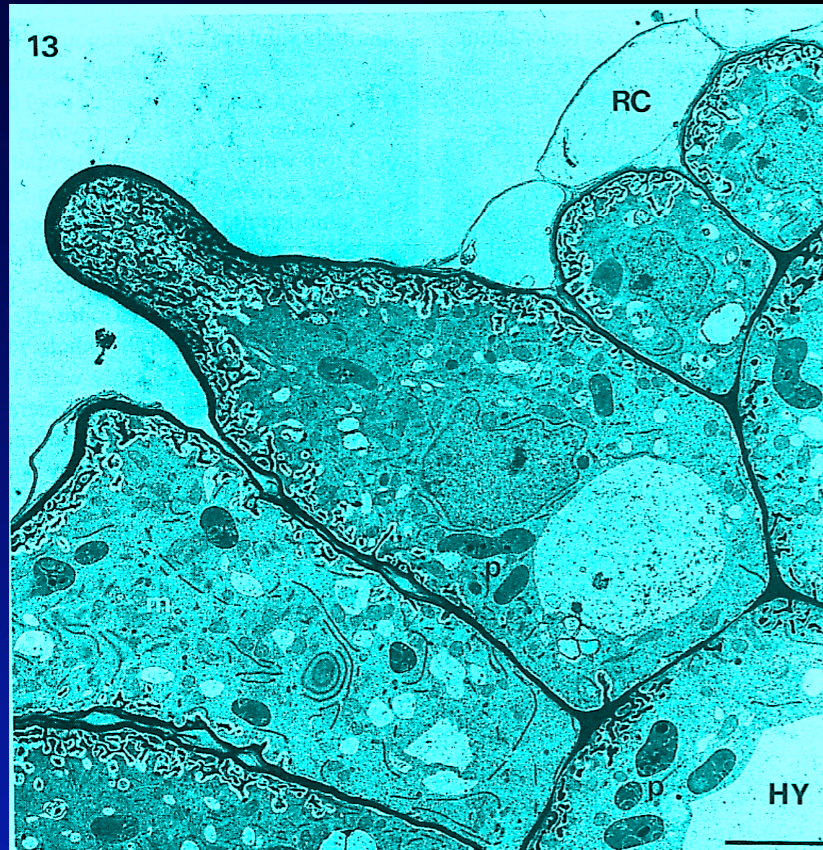


## Flavins in roots of Fe-deficient plants

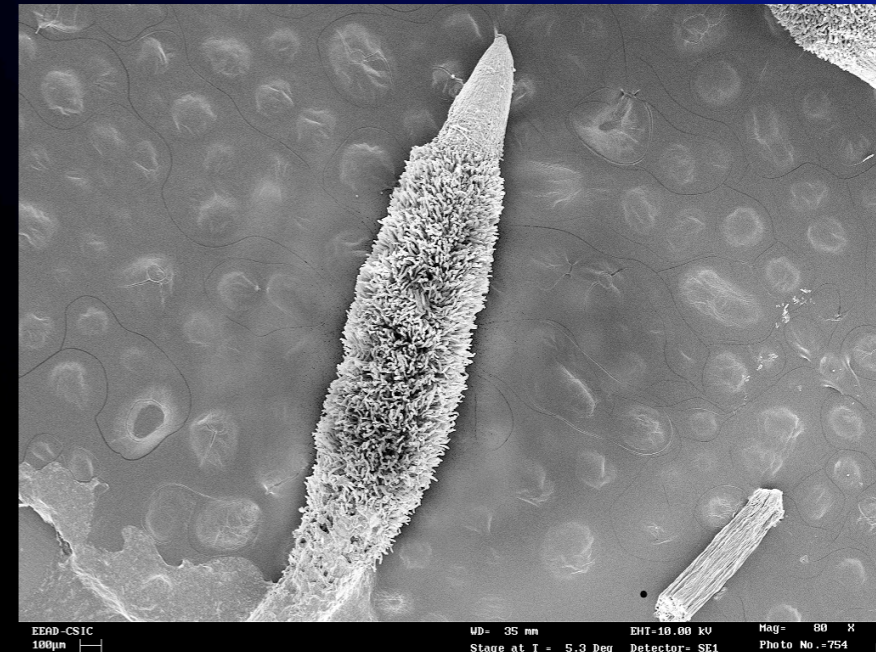
- Flavins in roots of Beta vulgaris
- Flavins in roots of Medicago truncatula
- Riboflavin biosynthesis
- Summary
  - ★ Current knowledge
  - ★ Possible roles



# Sugar beet (*Beta vulgaris* L.): a very efficient Strategy I species



Landsberg,  
JPN 1994



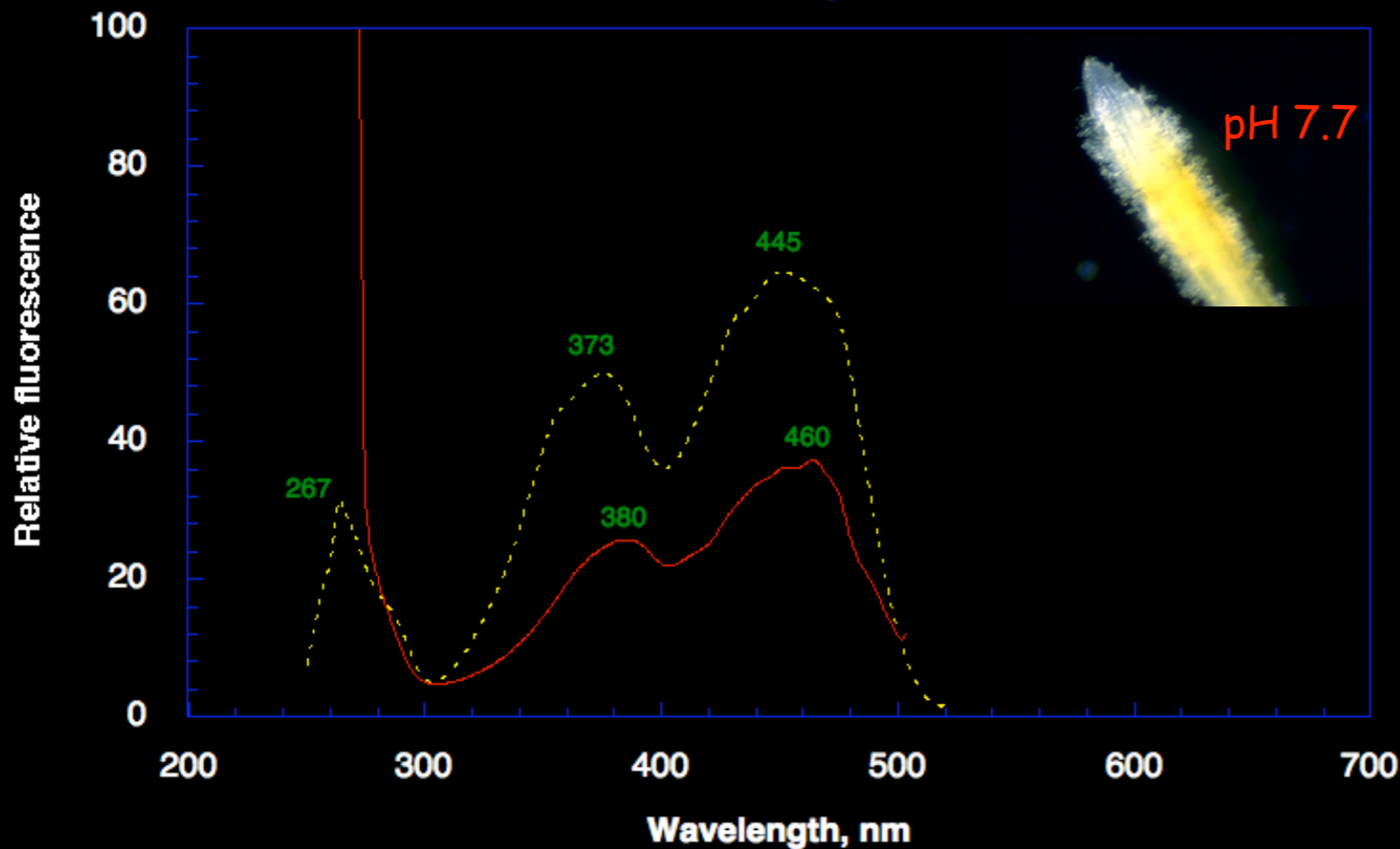
Susín PhD Thesis, 1994



Fe-deficient sugar beet (Beta vulgaris L.) roots have flavins



### Excitation Spectra



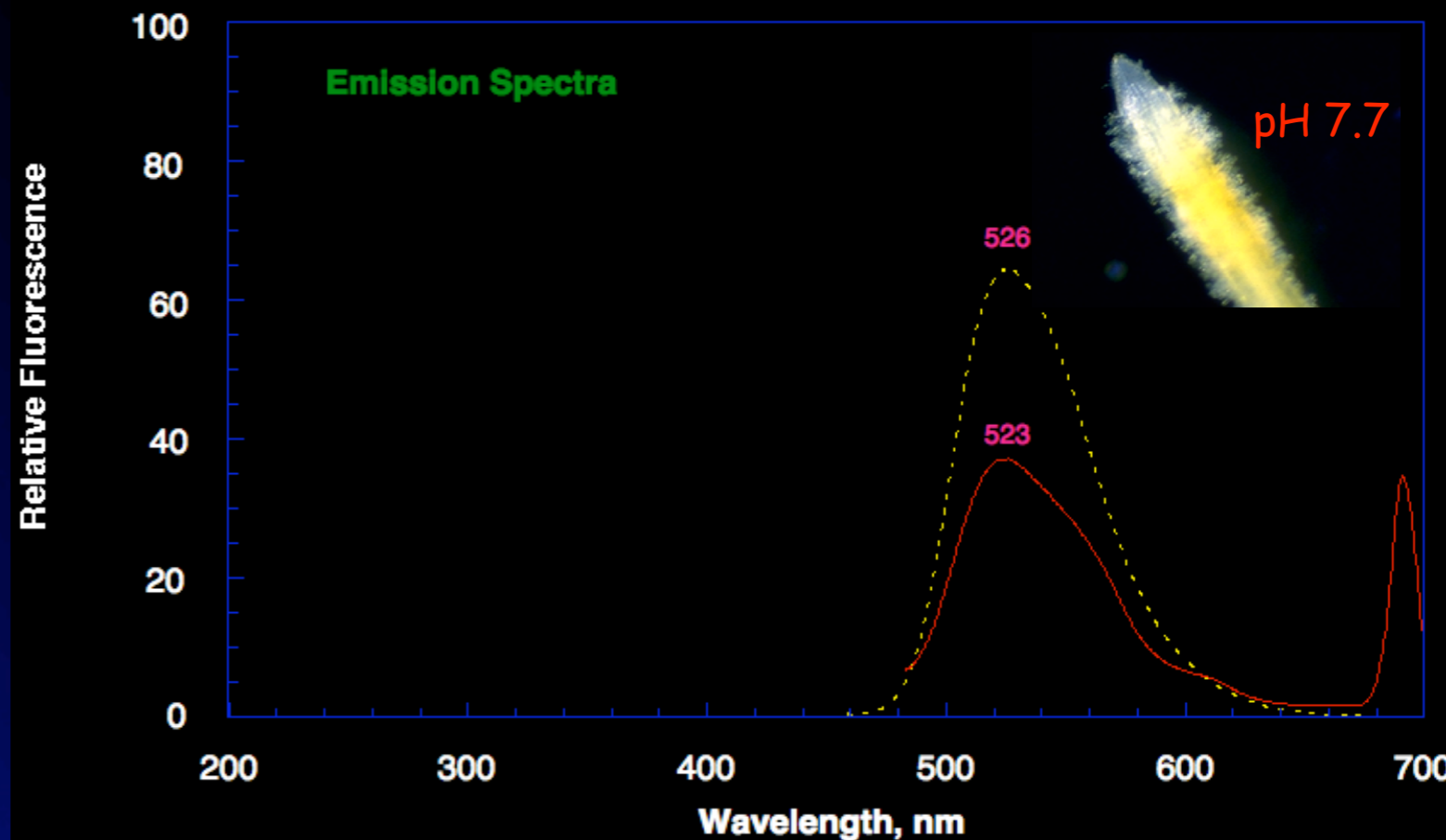
Flavins in Fe-deficient *Beta vulgaris*



Fe-deficient sugar beet (*Beta vulgaris* L.) roots have flavins

Susín et al., JBC 1993

### Emission Spectra





## Sugar beet (*Beta vulgaris* L.): which flavins?

riboflavin



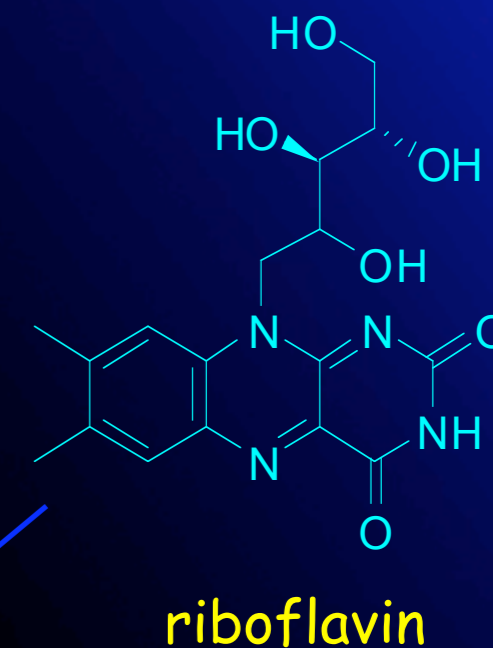
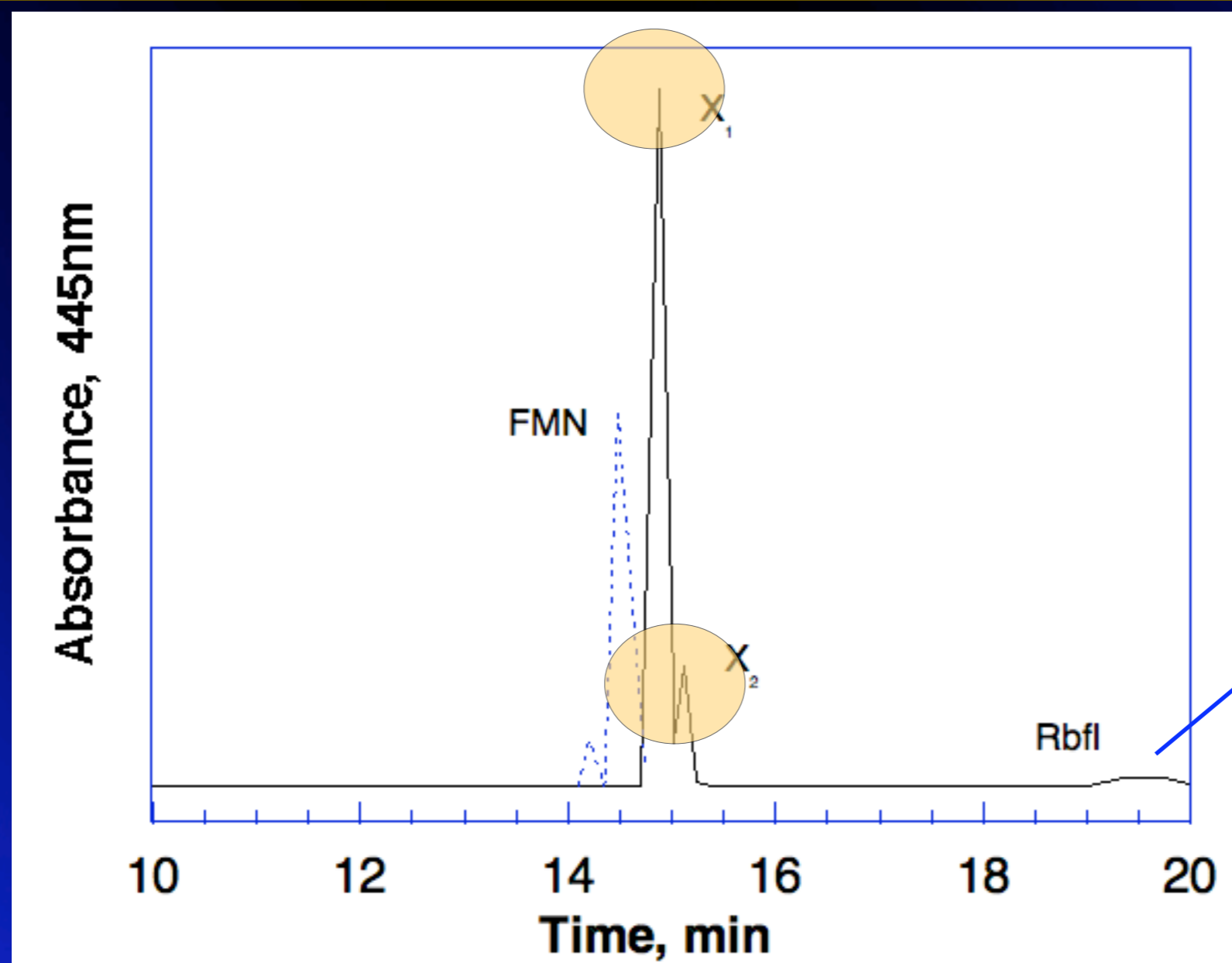
Susín et al., JBC 1993

Susín et al., Planta 1994





## Sugar beet (*Beta vulgaris* L.): which flavins?



Susín et al., JBC 1993







## Sugar beet (*Beta vulgaris* L.): which flavins?

### riboflavin sulphates

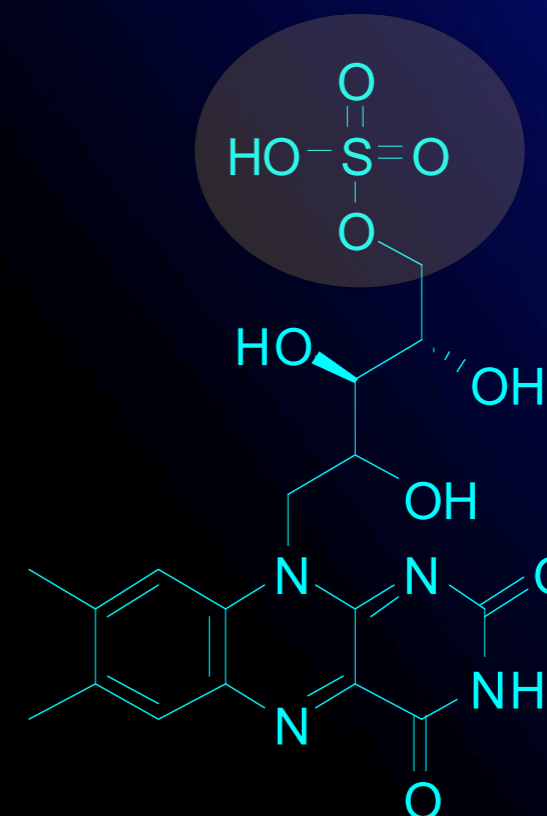
Susín et al., JBC 1993

#### 3'-sulphate riboflavin



Chemical Formula: C<sub>17</sub>H<sub>20</sub>N<sub>4</sub>O<sub>9</sub>S  
Exact Mass: 456,0951

#### 5'-sulphate riboflavin



Chemical Formula: C<sub>17</sub>H<sub>20</sub>N<sub>4</sub>O<sub>9</sub>S  
Exact Mass: 456,0951



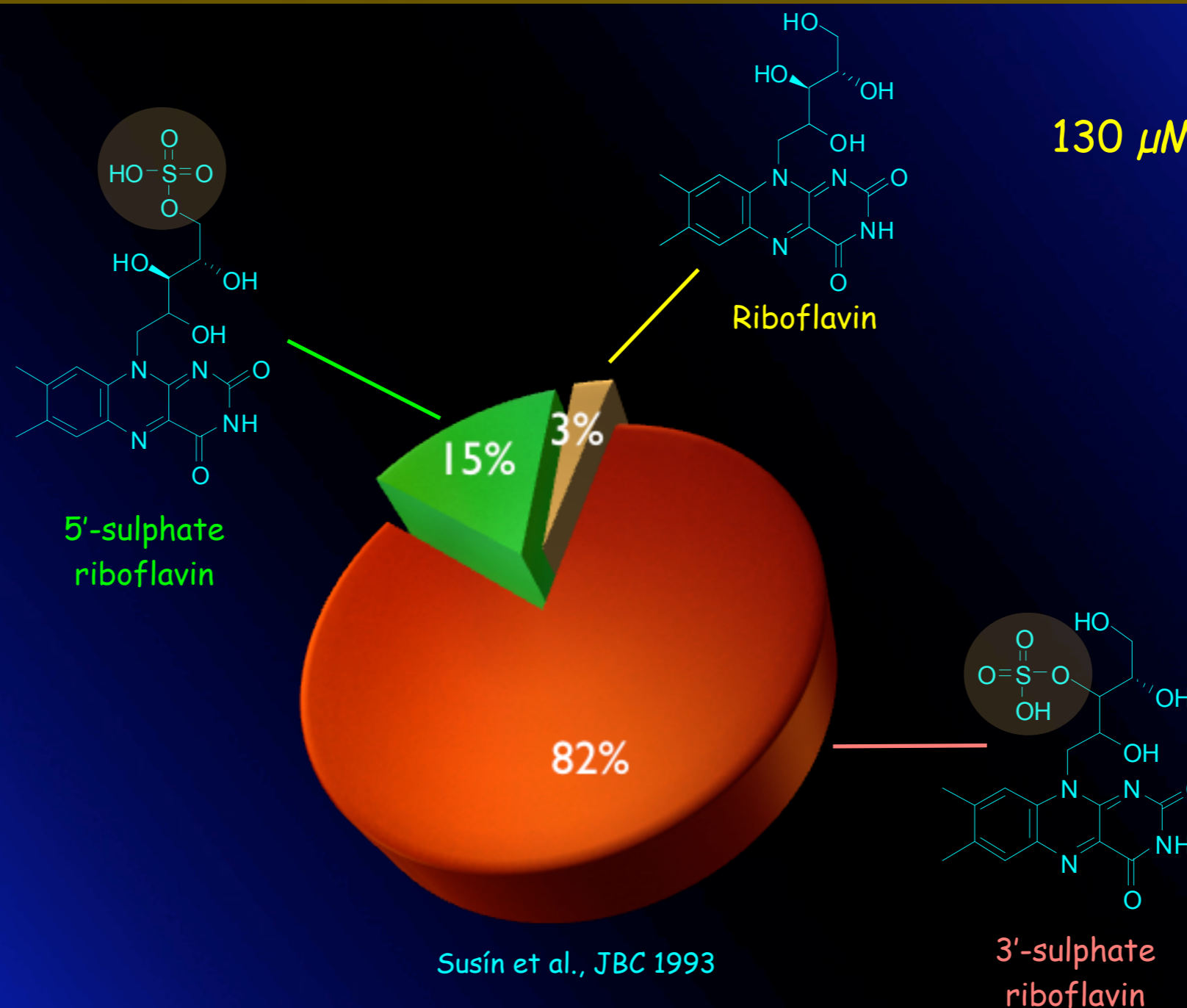


## Sugar beet (*Beta vulgaris* L.) flavin accumulation in roots

>1 mM

>20 mM

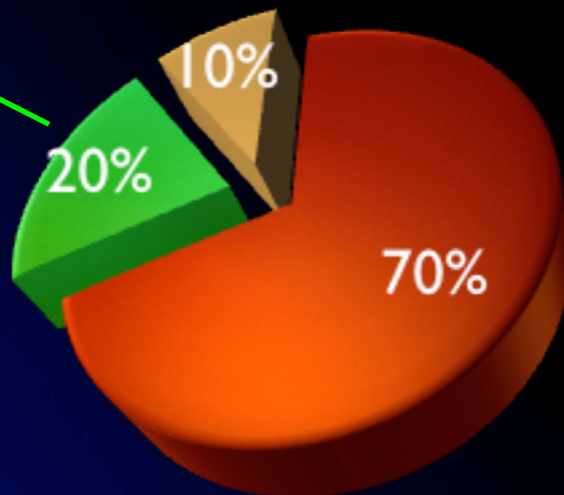
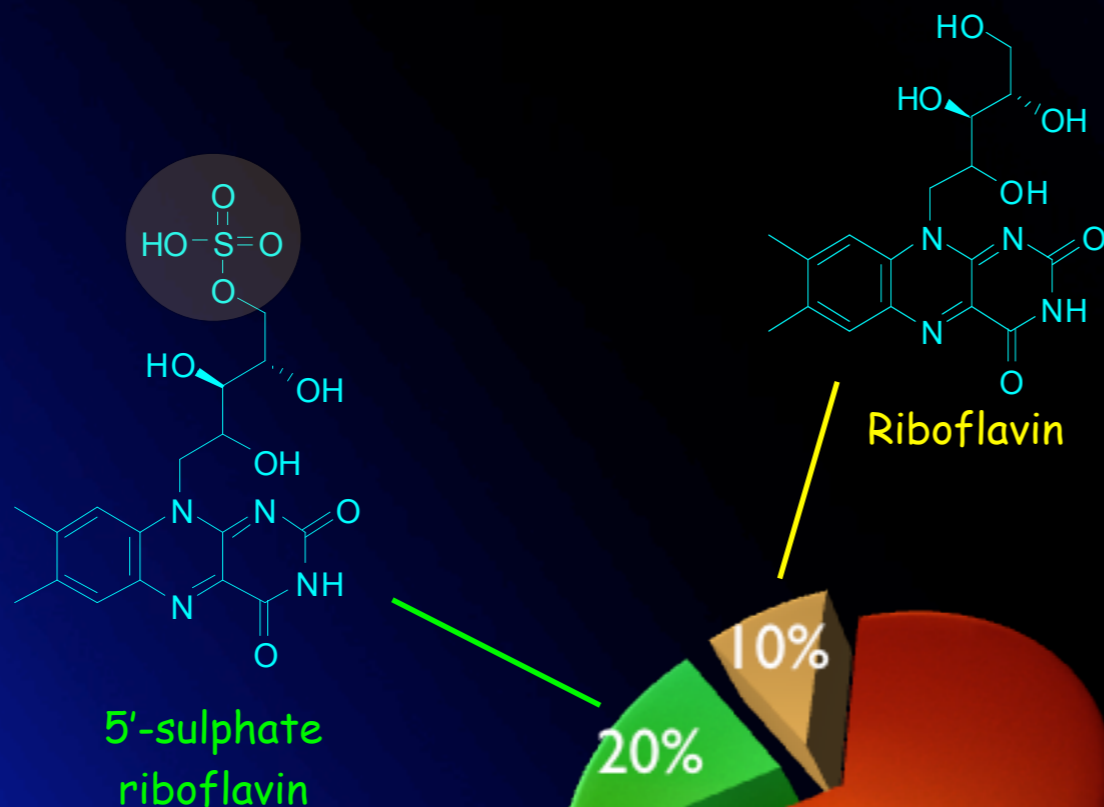
130  $\mu$ M



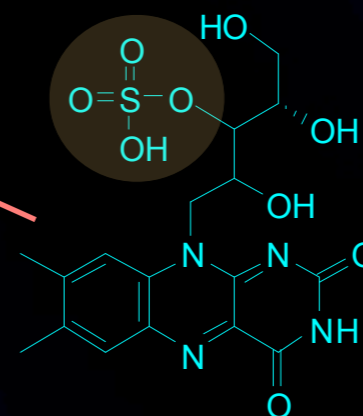
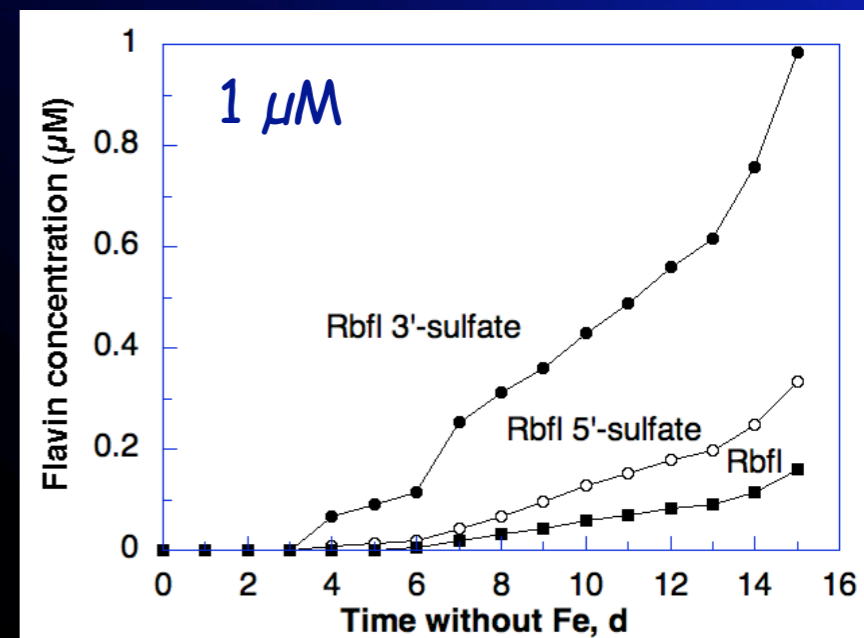
pH 5.5



# Sugar beet (*Beta vulgaris* L.) flavin excretion to the nutrient solution

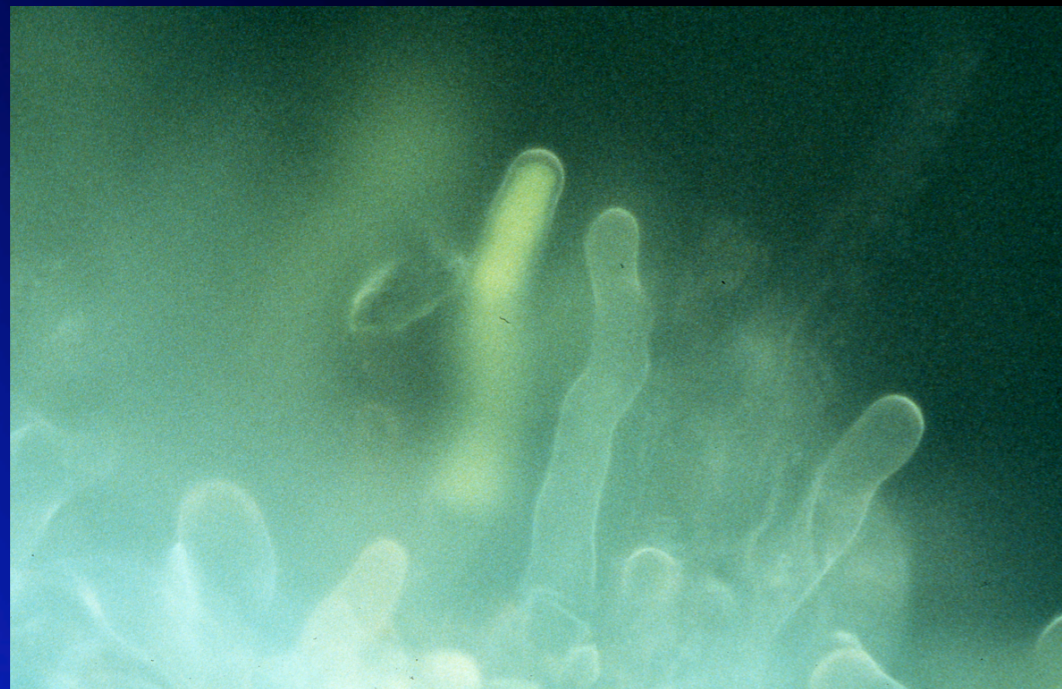


Susín et al., Planta 1994





Localization of flavins in sugar beet (*Beta vulgaris* L.) roots



Susín, PhD Thesis, 1994

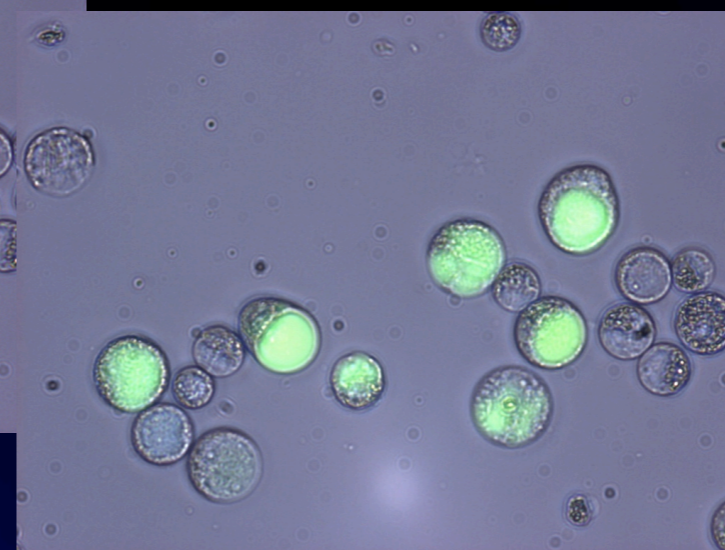
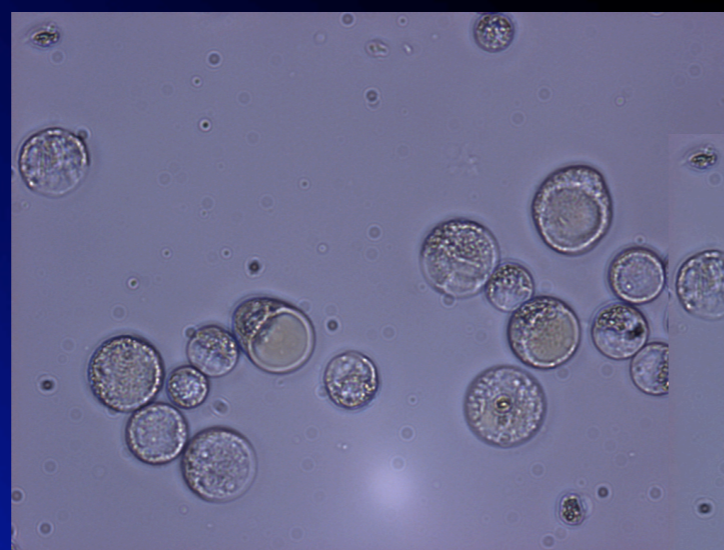
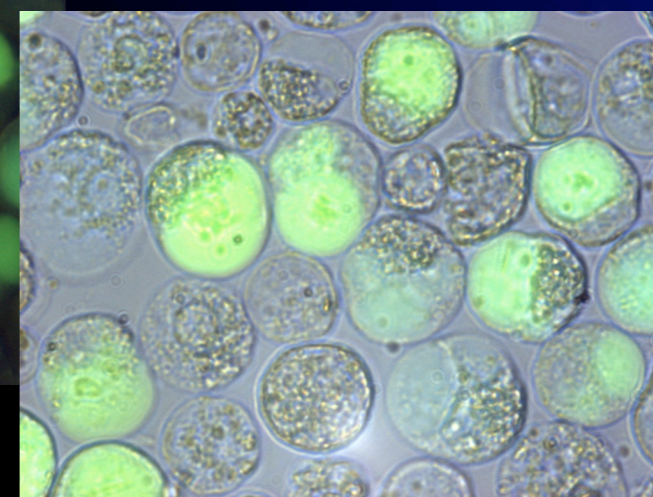
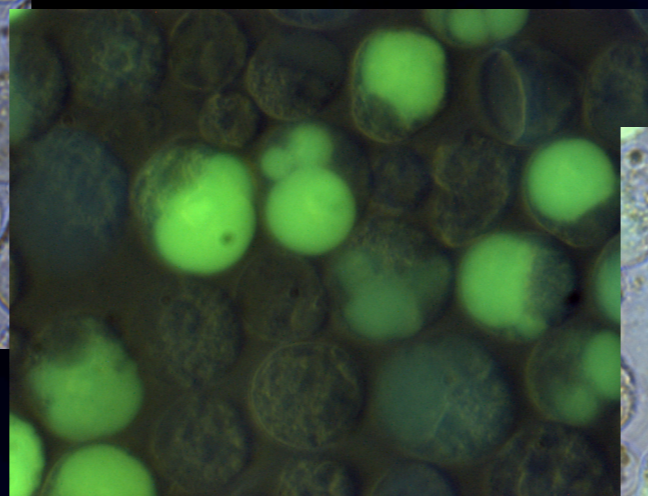
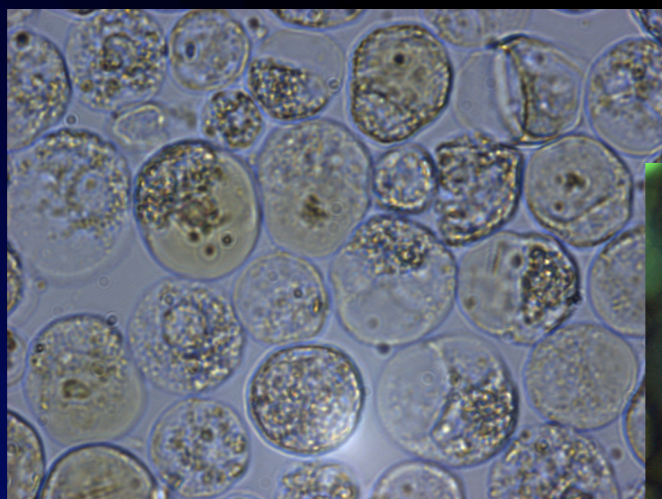




## Localization of flavins in sugar beet (*Beta vulgaris* L.) roots

### Root protoplasts

Zaharieva et al., in preparation

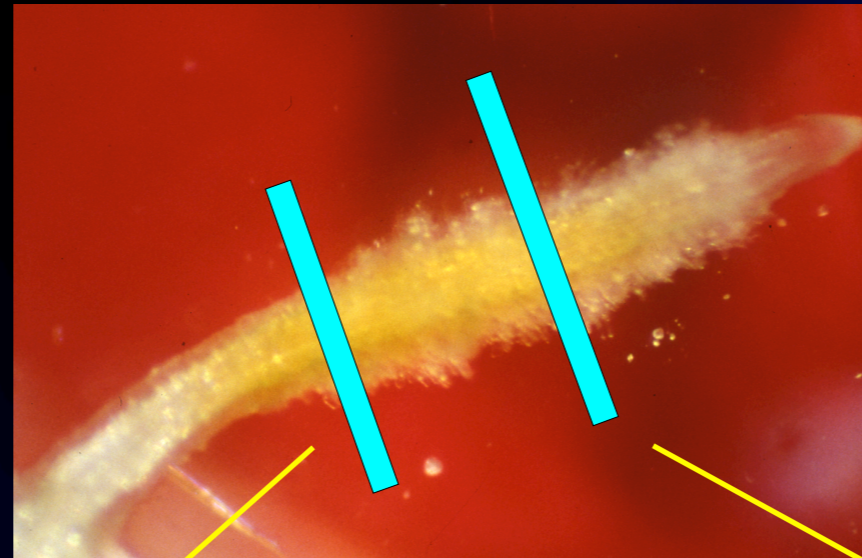


Most of the flavins are in the vacuole

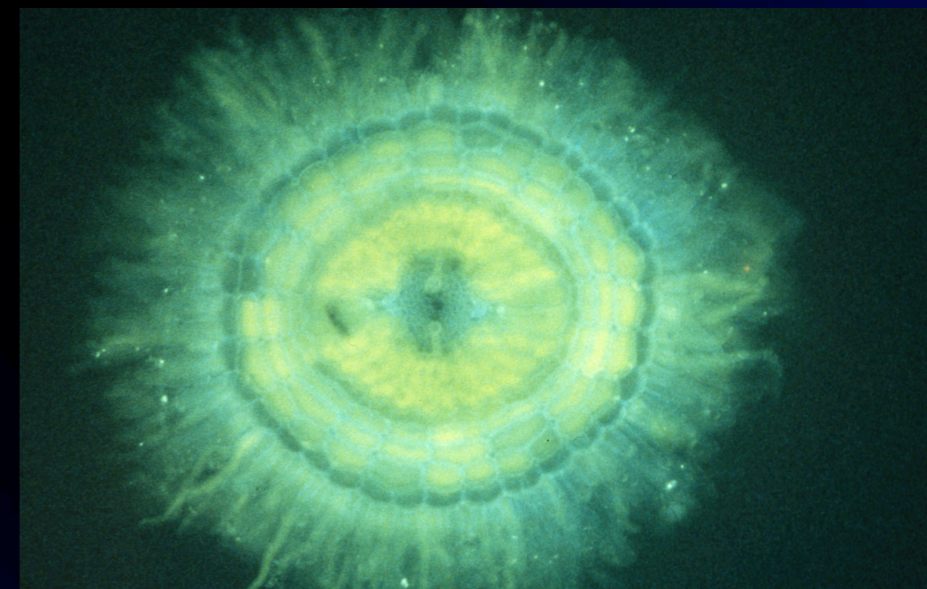
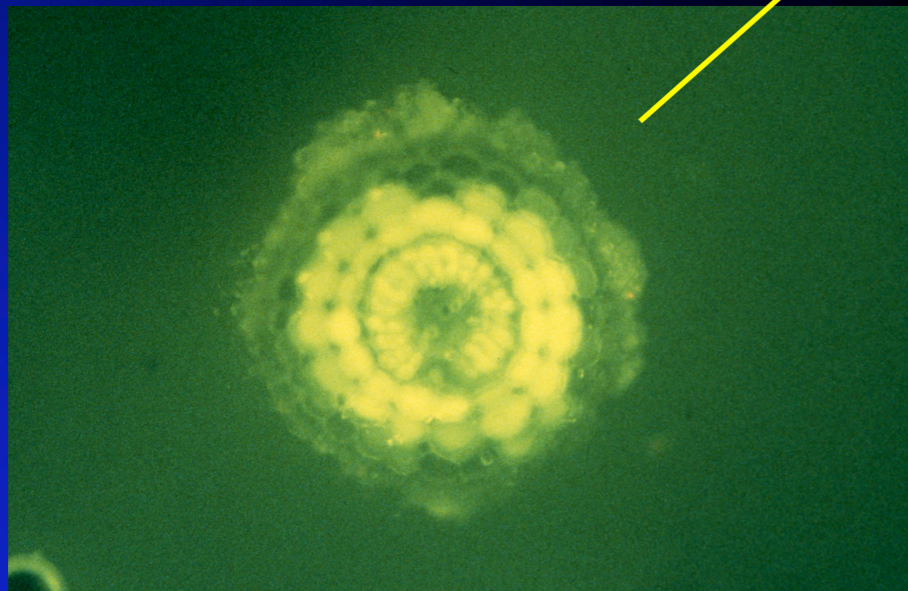




## Localization of flavins in sugar beet (*Beta vulgaris* L.)



Susín, PhD Thesis, 1994





## Fe-deficient Medicago truncatula



+ Fe

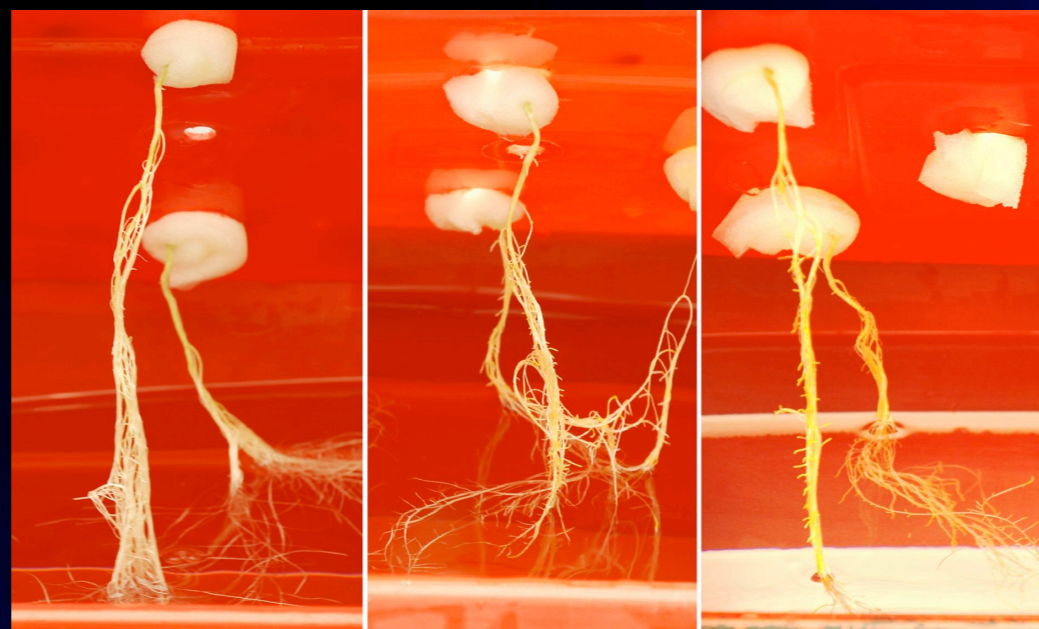
- Fe, pH 5.5

- Fe, pH 8.0



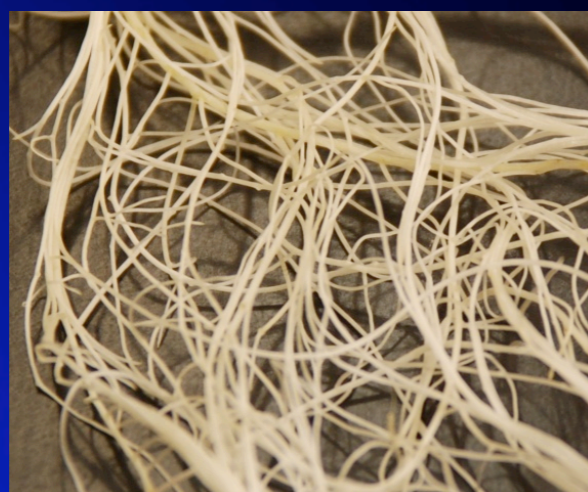


## Fe-deficient Medicago truncatula roots have flavins



+ Fe

- Fe, pH 5.5 - Fe, pH 8.0



+ Fe



- Fe, pH 5.5



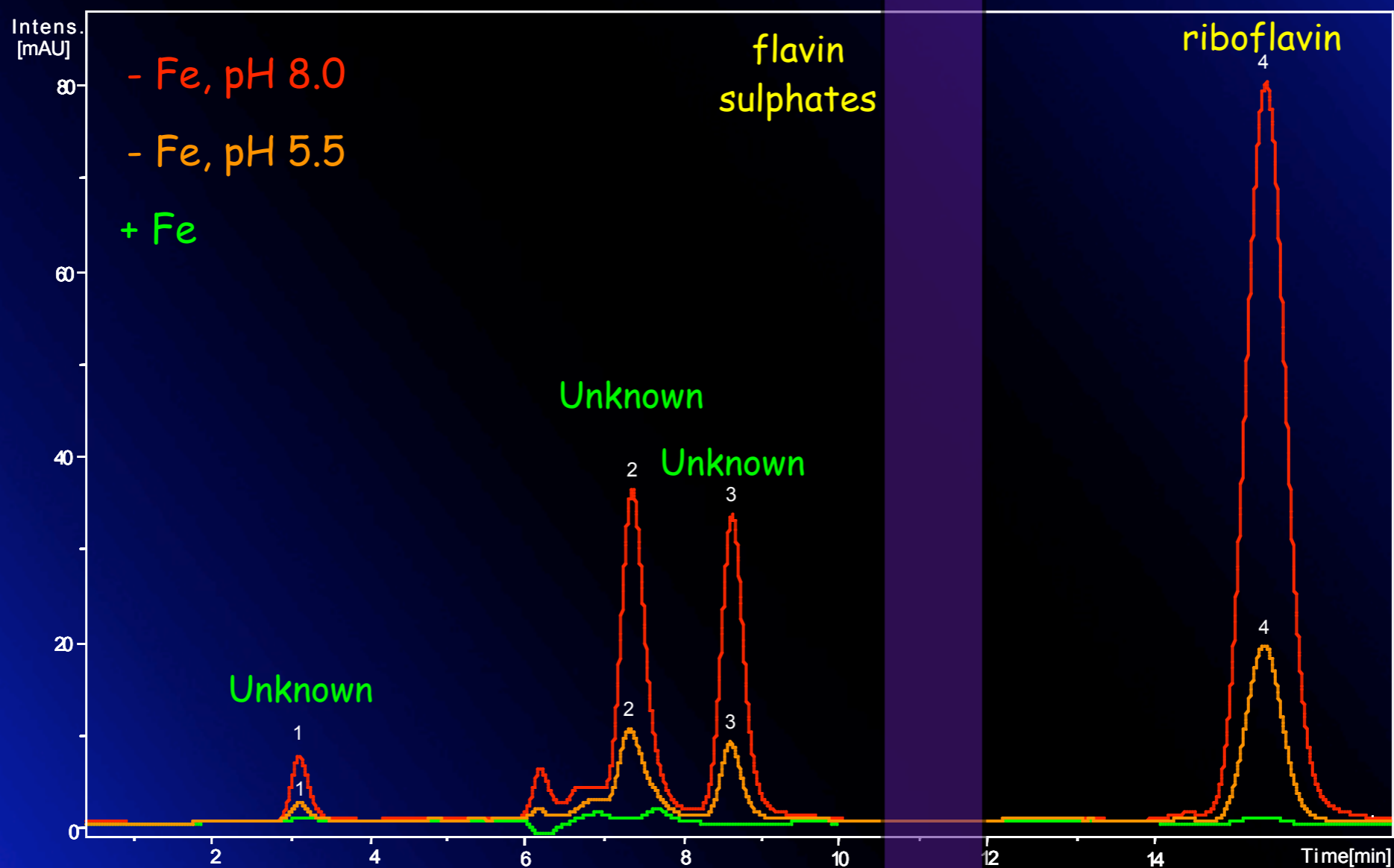
- Fe, pH 8.0





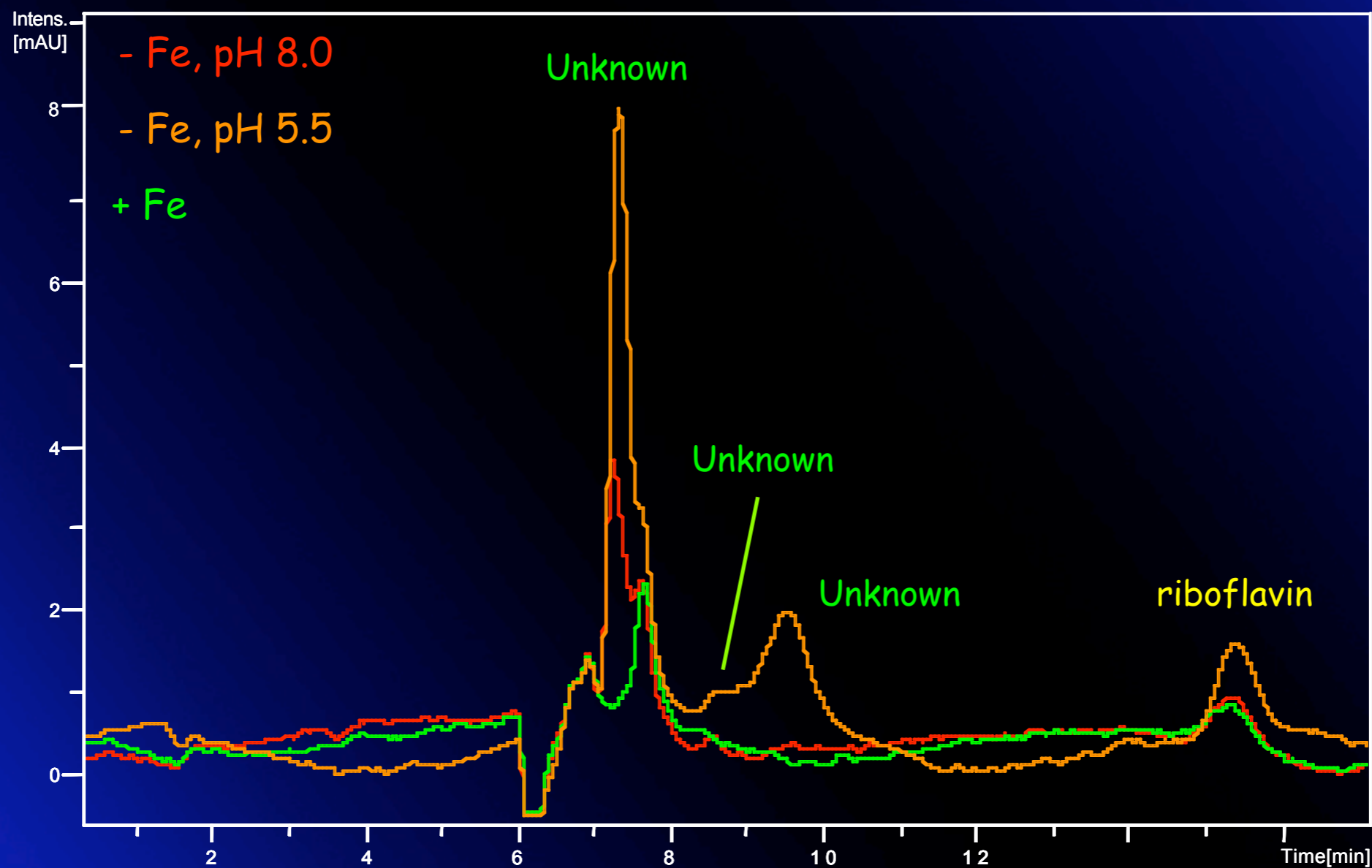


## Flavin compounds in root extracts of *Medicago truncatula*





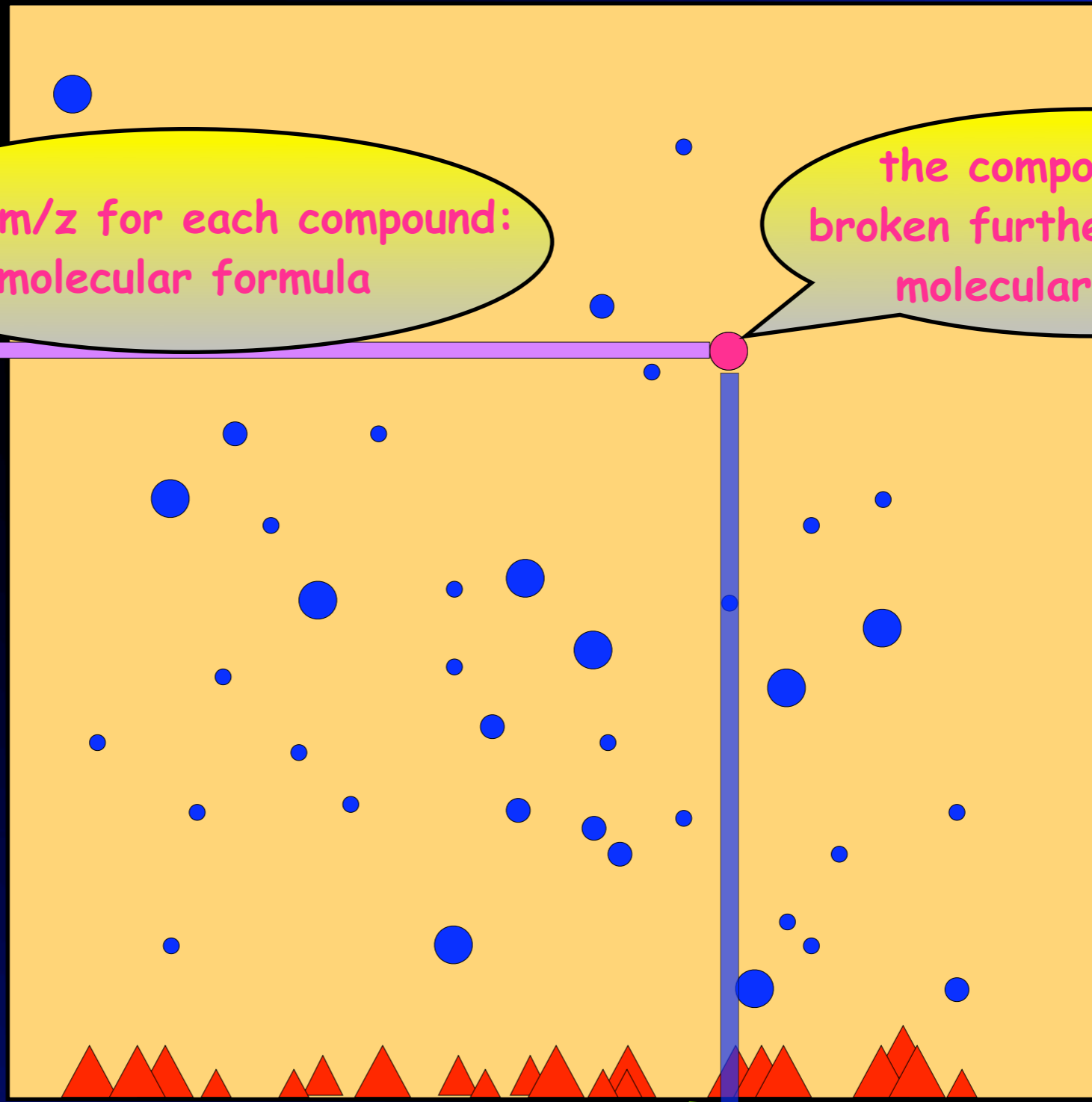
## Flavin compounds in nutrient solutions of *Medicago truncatula*



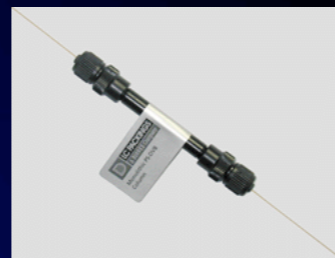
unique m/z for each compound:  
molecular formula

the compound can be  
broken further by MS/MS:  
molecular structure

mass/charge ratio  
MS (ESI/TOF)

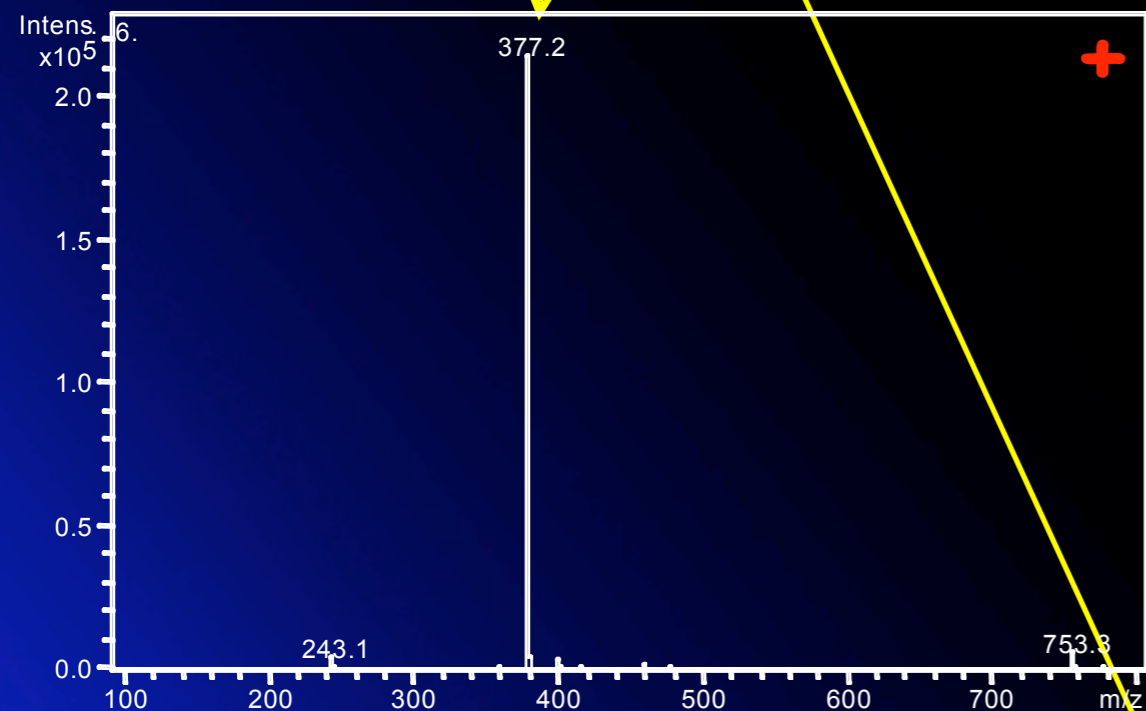
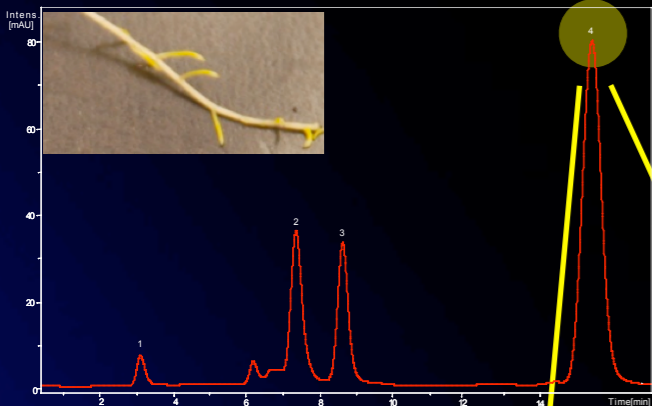


Time (HPLC)

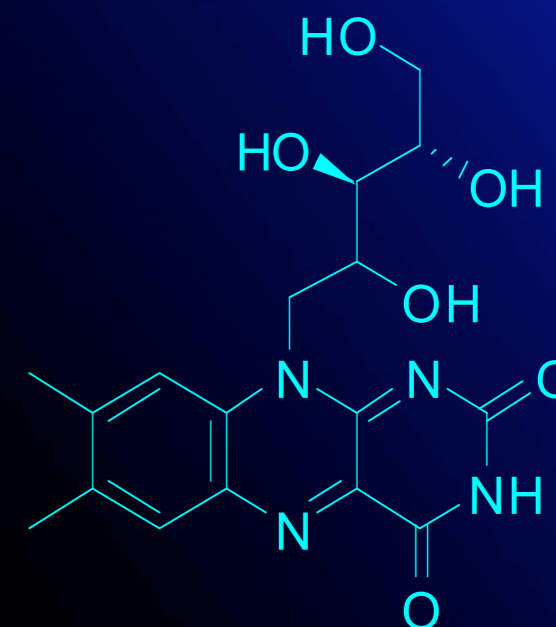




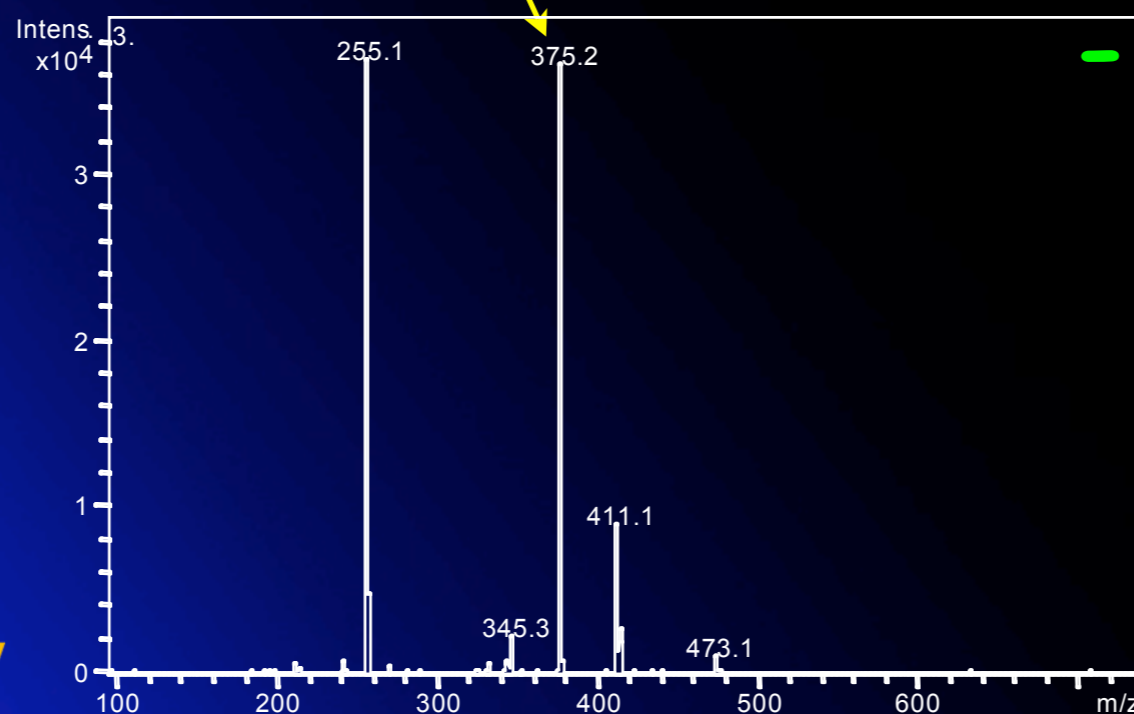
## Identification by HPLC-ESI-MS(TOF)



Chemical Formula: C<sub>17</sub>H<sub>20</sub>N<sub>4</sub>O<sub>6</sub>  
Exact Mass: 376,1383

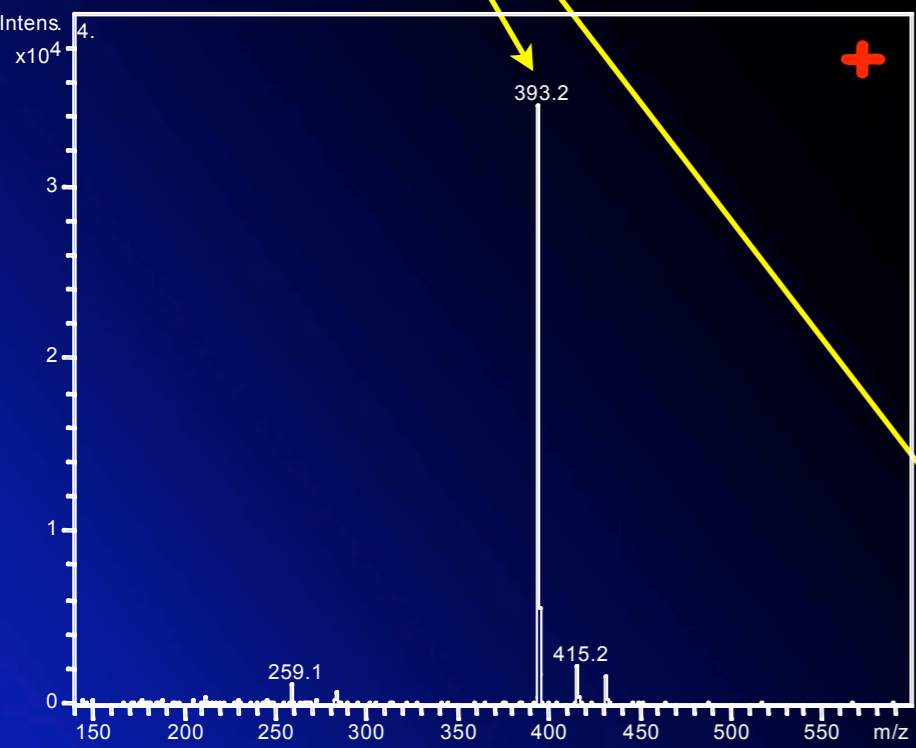
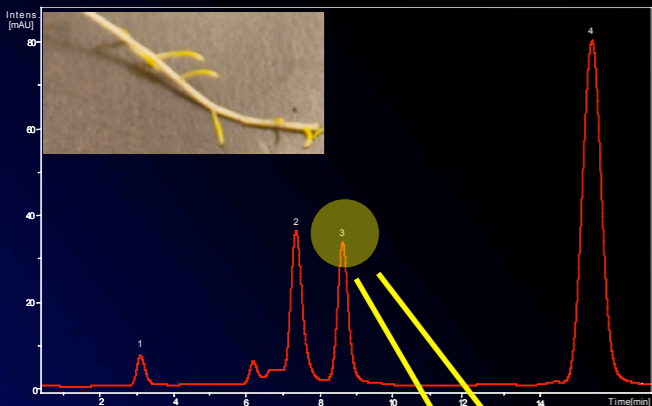


riboflavin

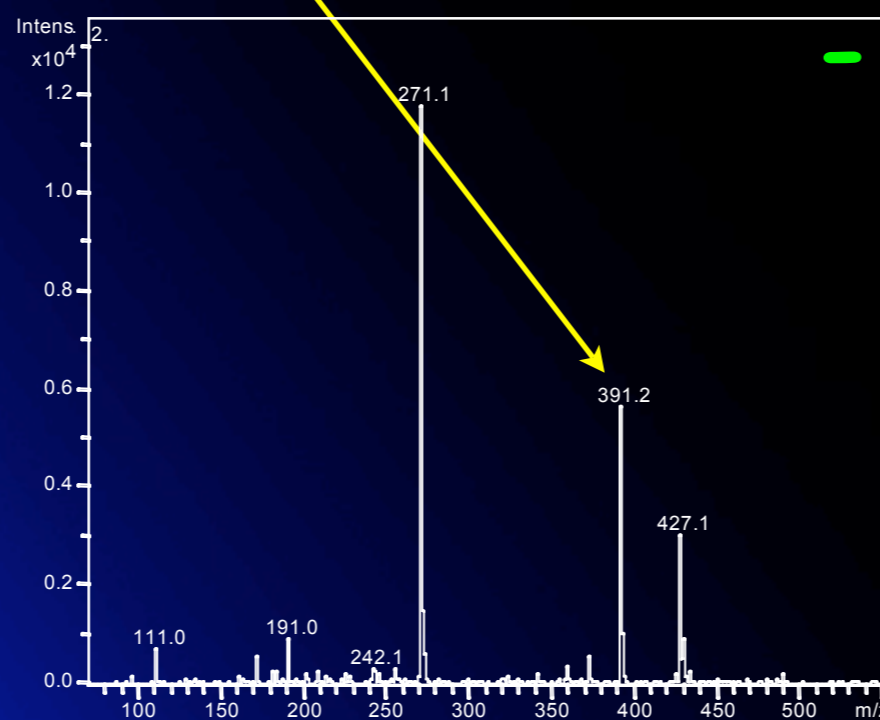
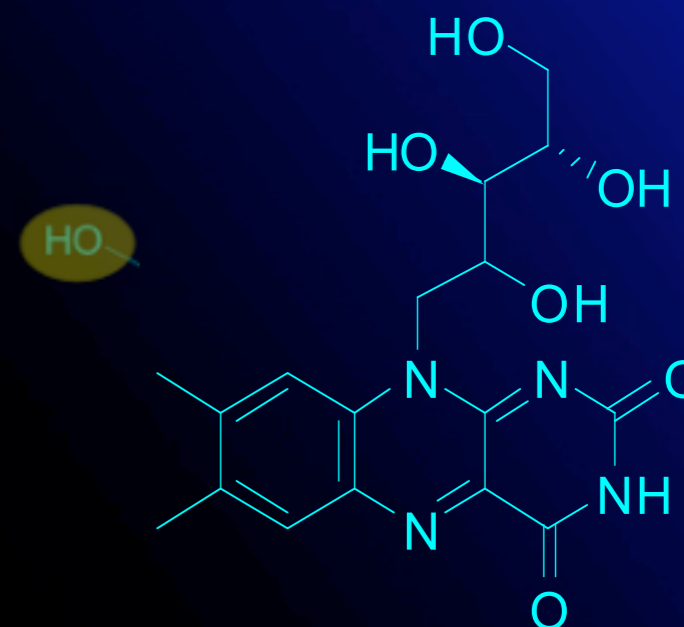




## Identification by HPLC-ESI-MS(TOF)

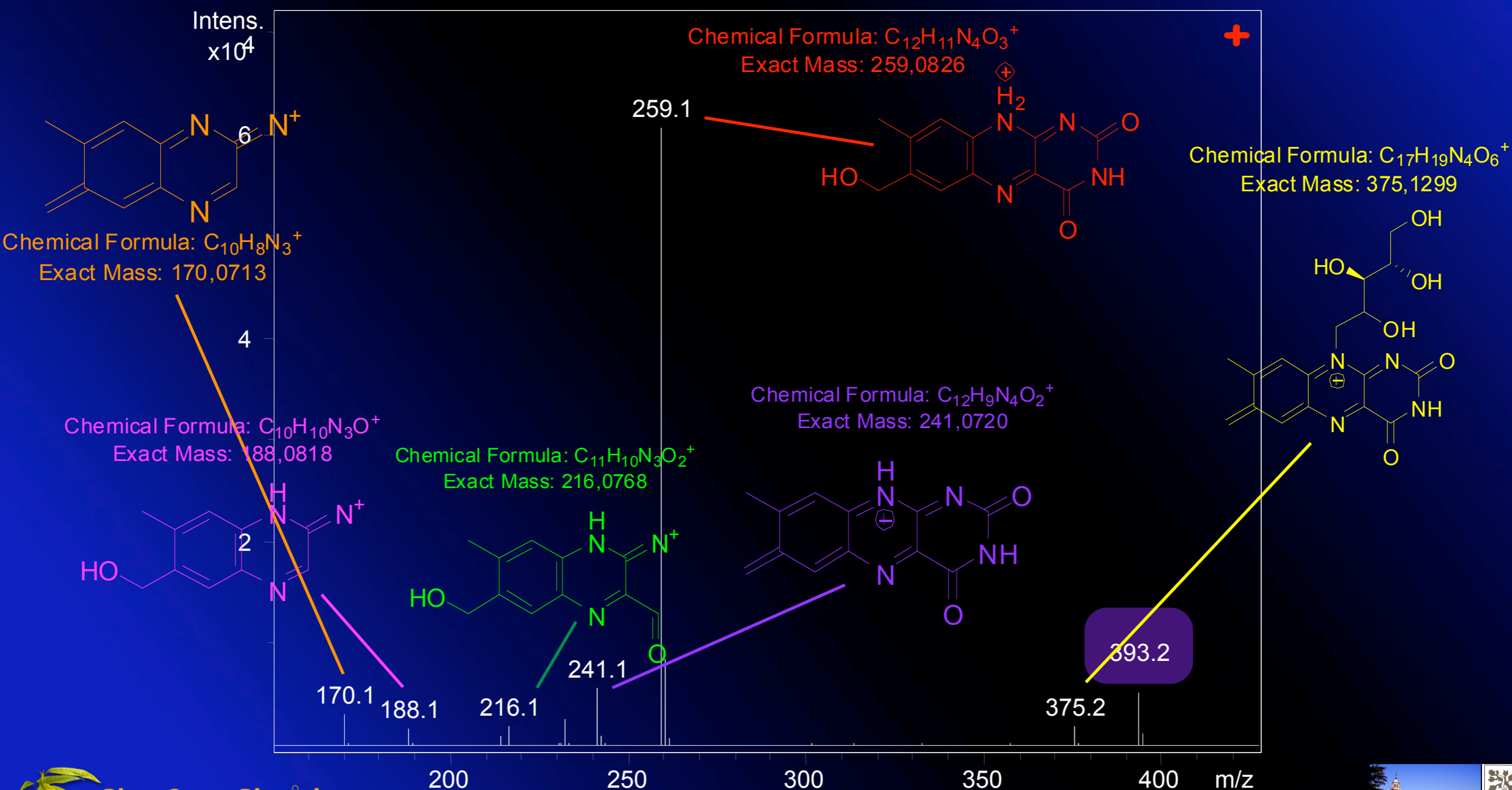
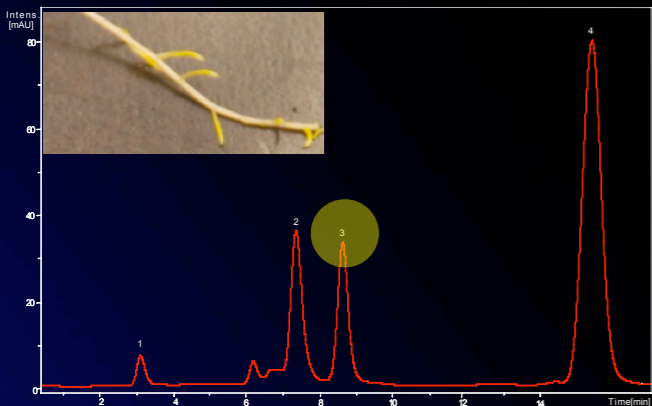


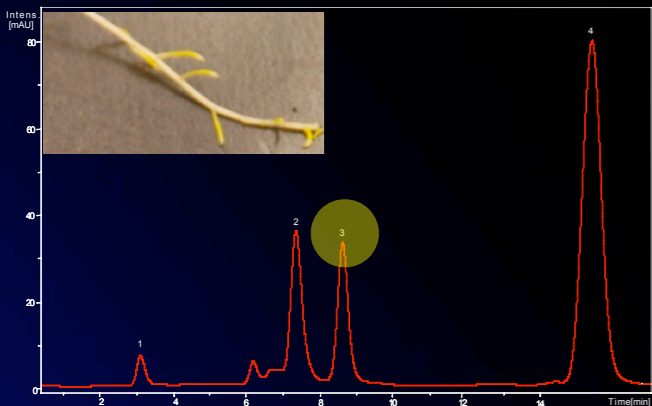
Chemical Formula: C<sub>17</sub>H<sub>20</sub>N<sub>4</sub>O<sub>7</sub>  
Exact Mass: 392,1332



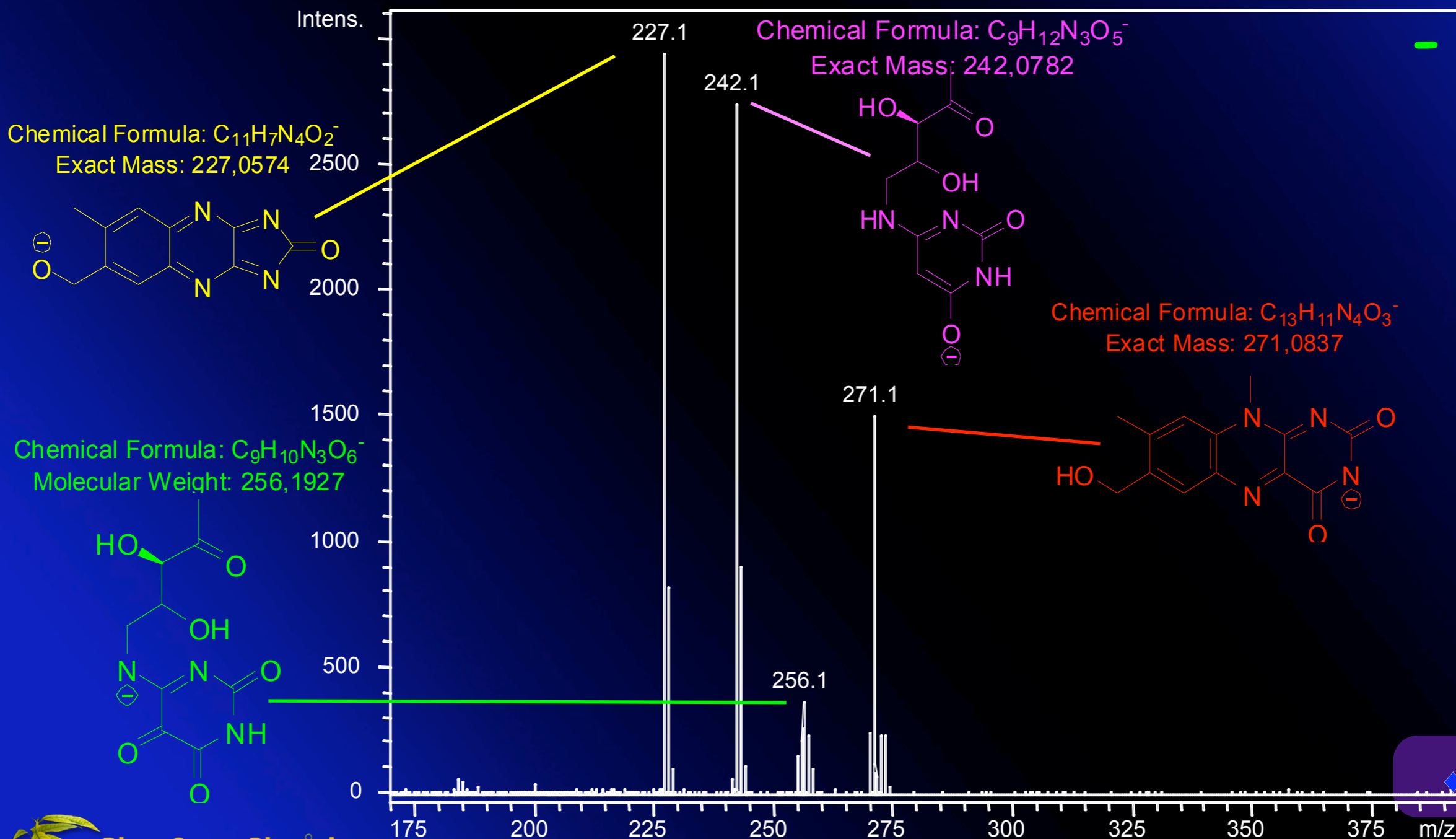


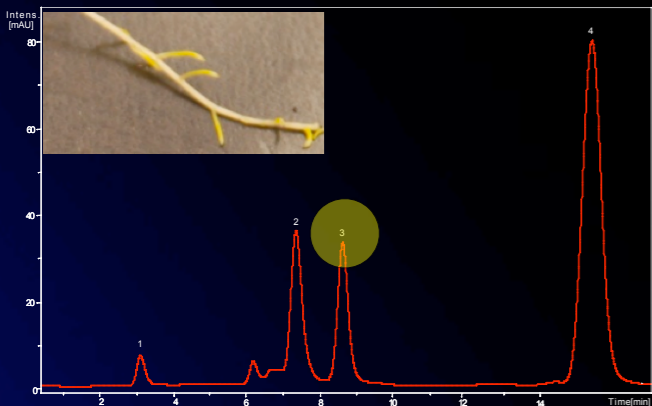
# Identification by HPLC-ESI-MS/MS(Q/TOF)





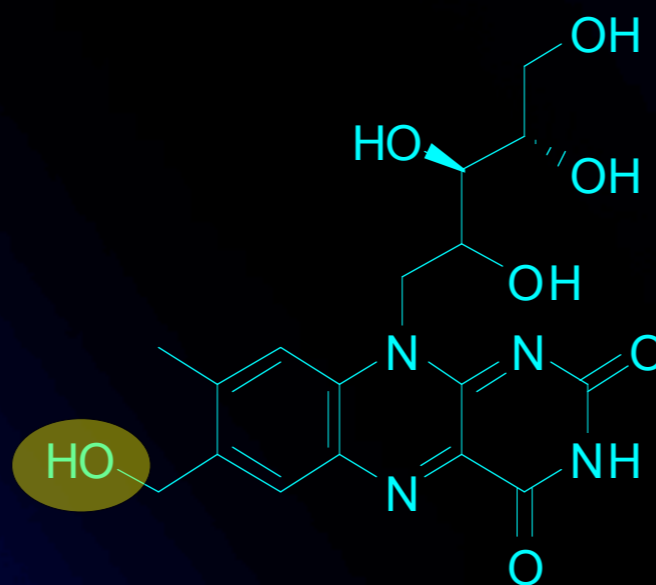
## Identification by HPLC-ESI-MS/MS(Q/TOF)





## Identification by HPLC-ESI-MS/MS(Q/TOF)

Chemical Formula: C<sub>17</sub>H<sub>20</sub>N<sub>4</sub>O<sub>7</sub>  
Exact Mass: 392,1332

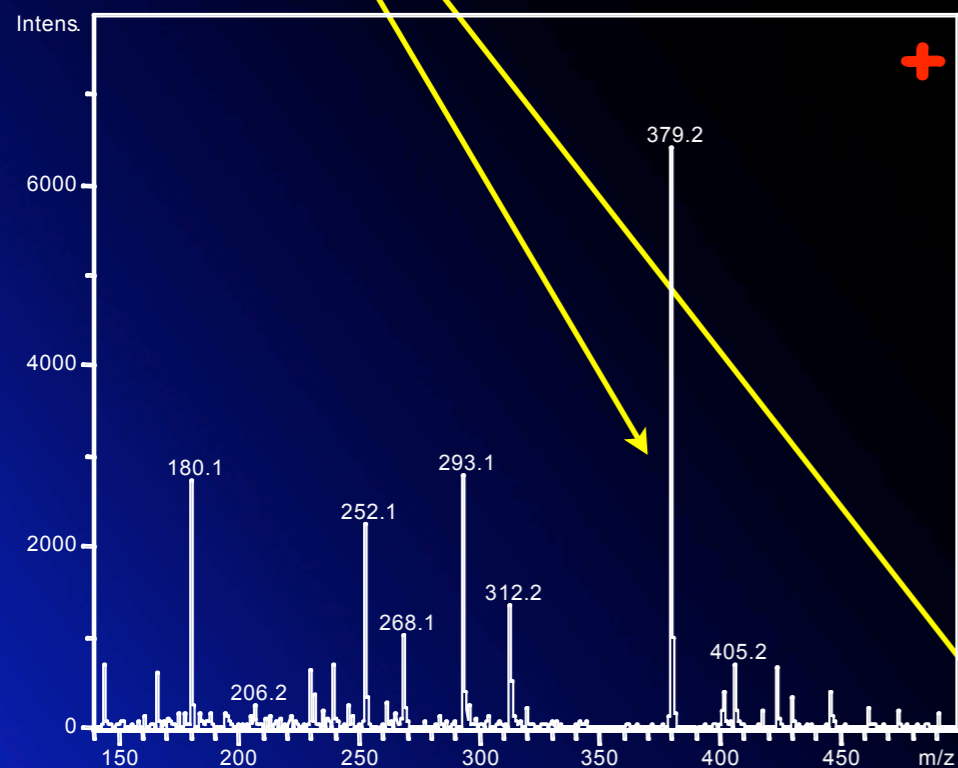
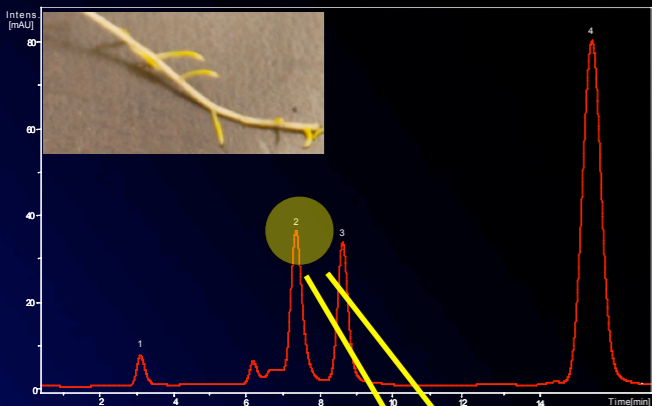


7- $\alpha$  hidroxy riboflavin

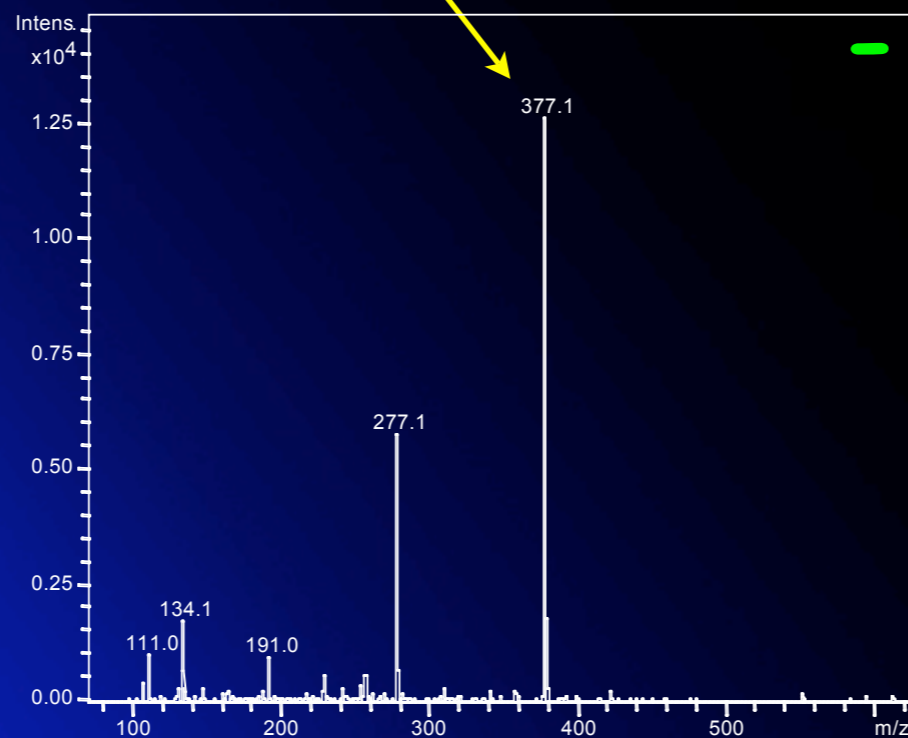


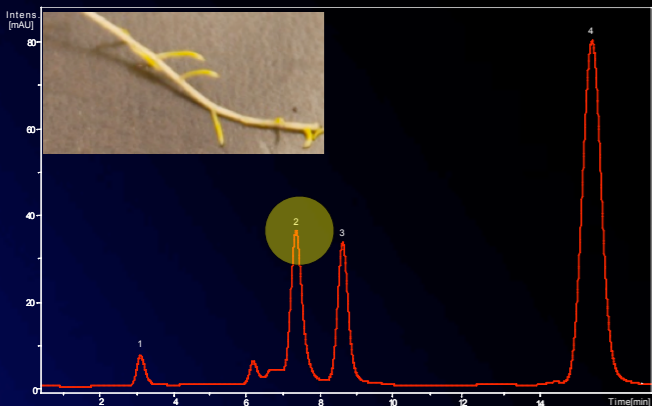


# Identification by HPLC-ESI-MS(TOF)

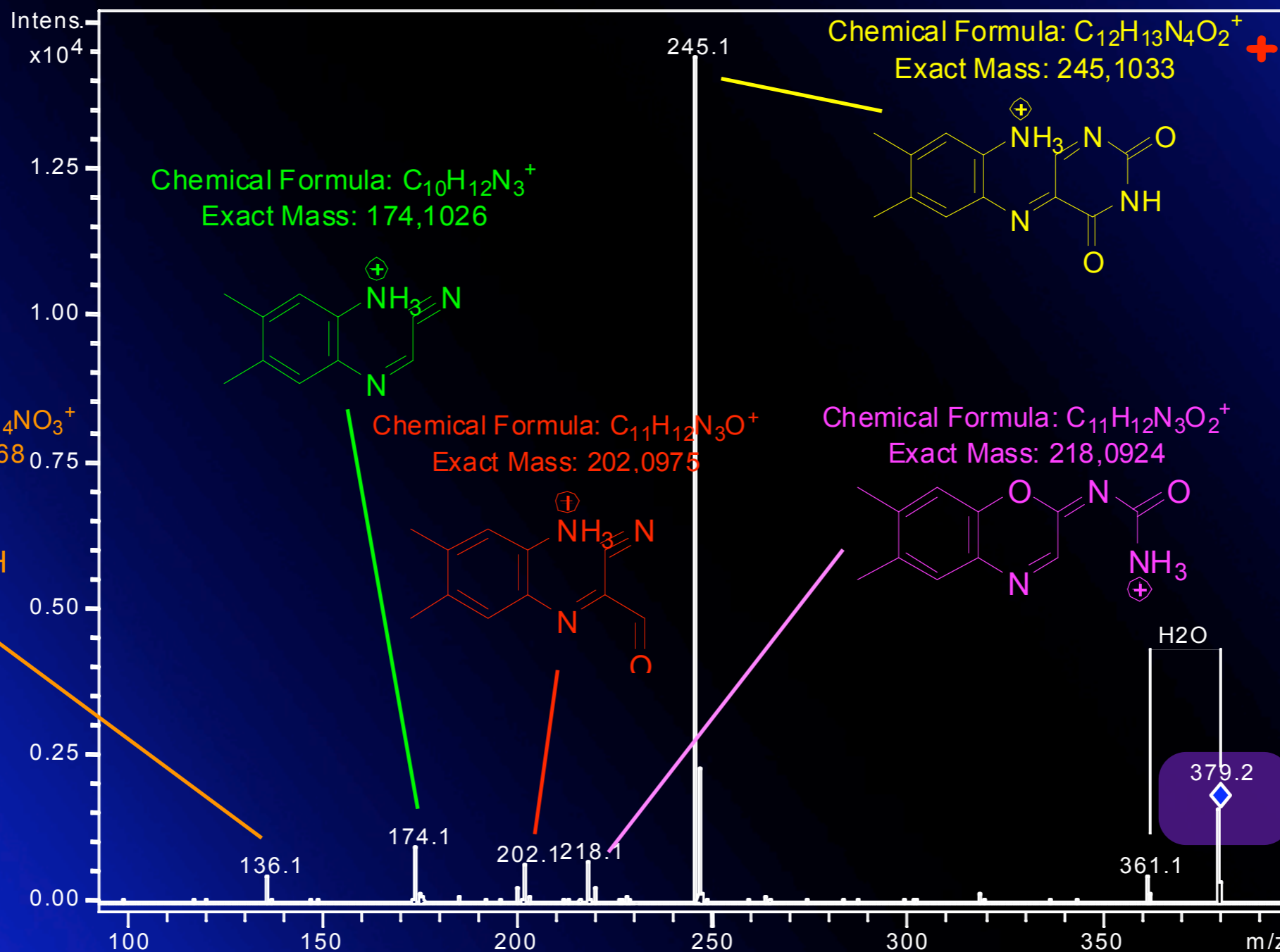


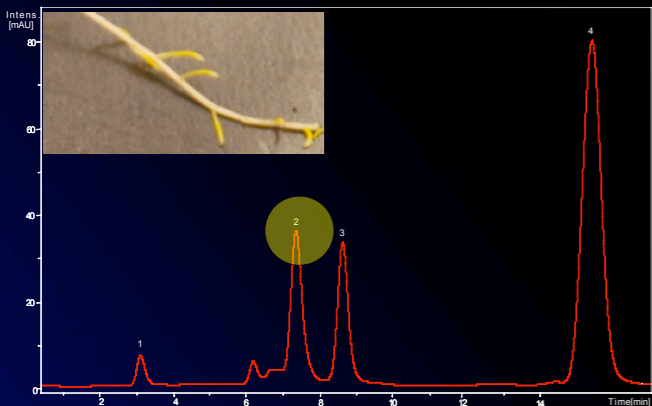
Chemical Formula: C<sub>17</sub>H<sub>22</sub>N<sub>4</sub>O<sub>6</sub>  
Exact Mass: 378,1539



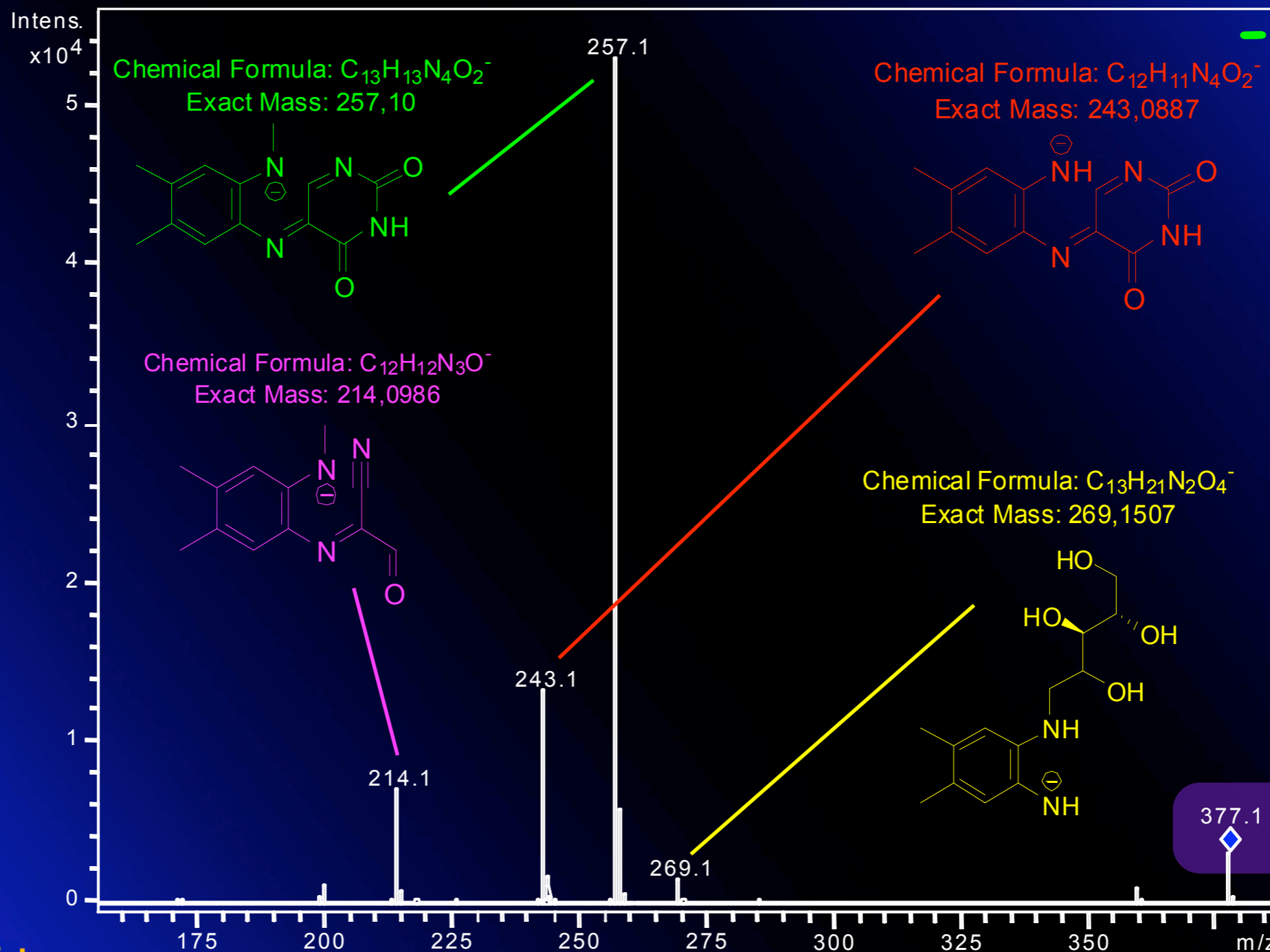


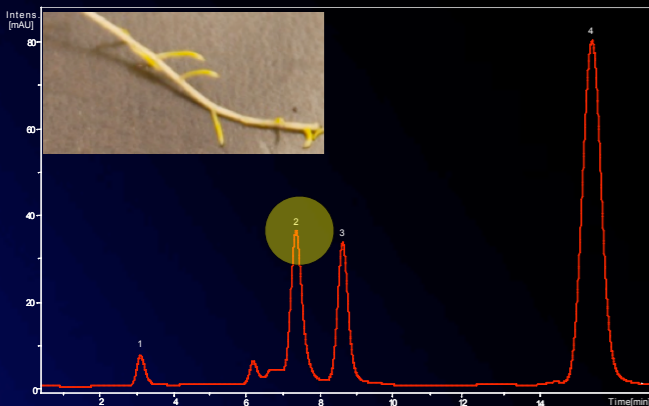
## Identification by HPLC-ESI-MS/MS(Q/TOF)



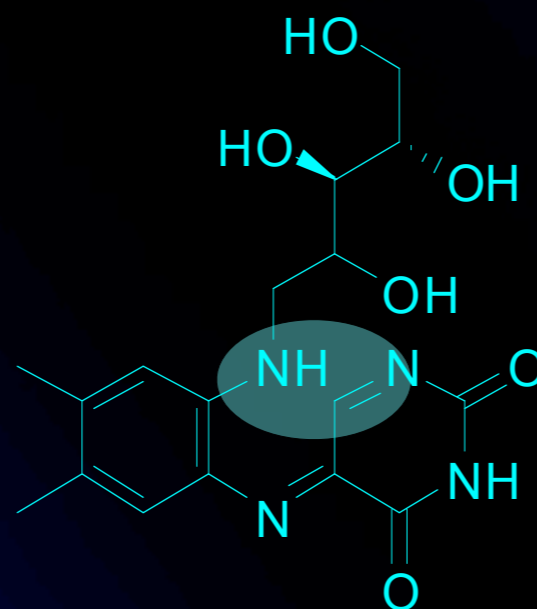


# Identification by HPLC-ESI-MS/MS(Q/TOF)





## Identification by HPLC-ESI-MS/MS(Q/TOF)

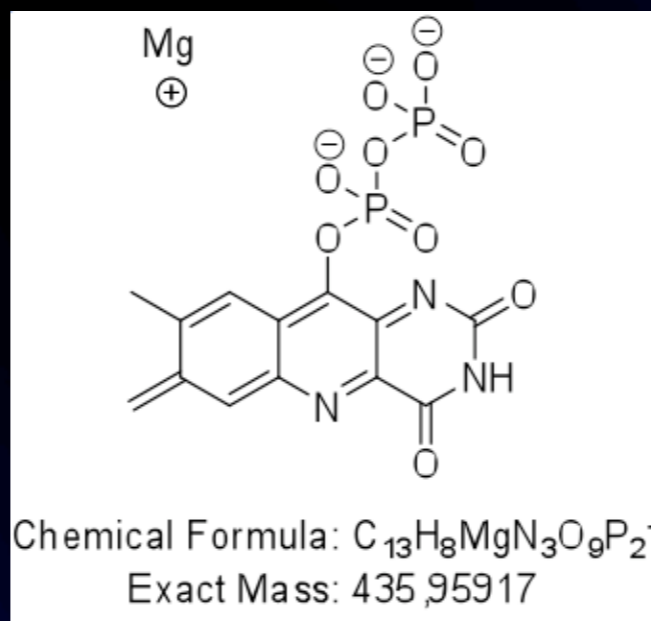
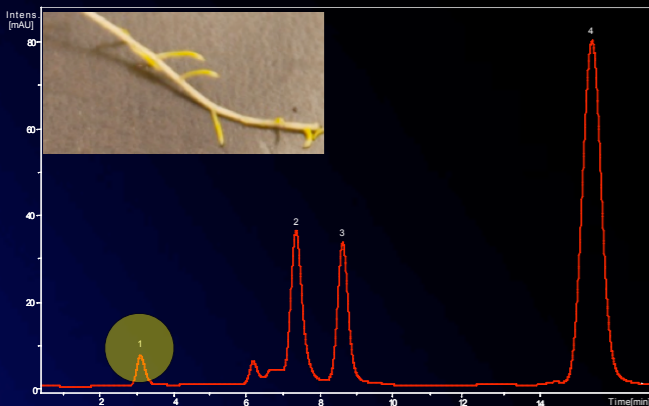


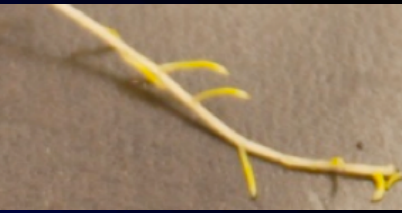
(E)-5-(4,5-dimethyl-2-((3R,4S)-2,3,4,5-tetrahydroxypentylamino)phenylimino)pyrimidine-2,4(3H,5H)-dione





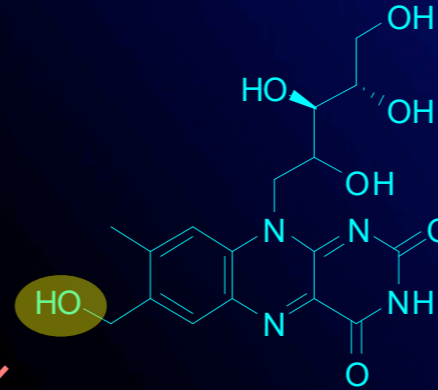
## Identification by HPLC-ESI-MS/MS(Q/TOF)



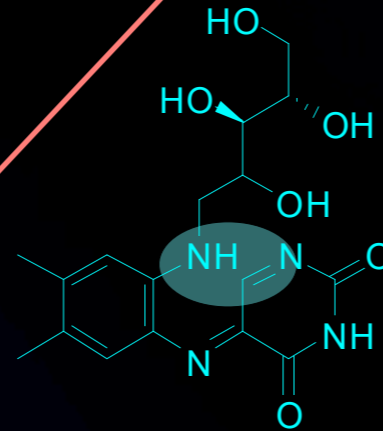


# Medicago truncatula flavin accumulation in roots

Flavins in Fe-deficient *Medicago truncatula*



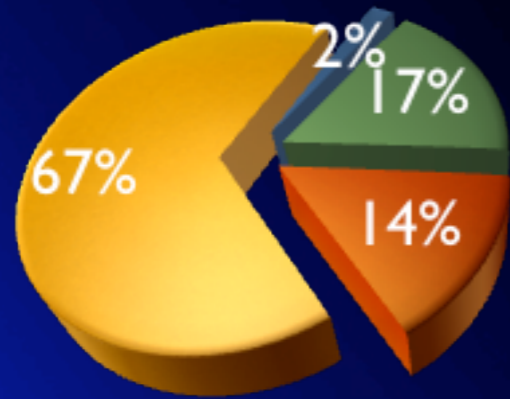
7-alpha hidroxy riboflavin



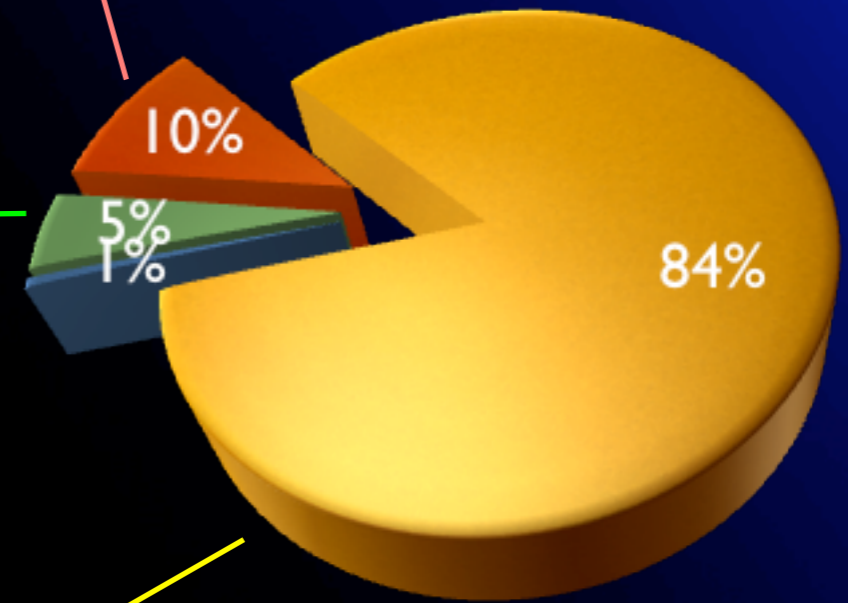
(E)-5-(4,5-dimethyl-2-((3R,4S)-2,3,4,5-tetrahydroxypentylamino)phenylimino) pyrimidine-2,4(3H,5H)-dione



Riboflavin



- Fe, pH 5.5

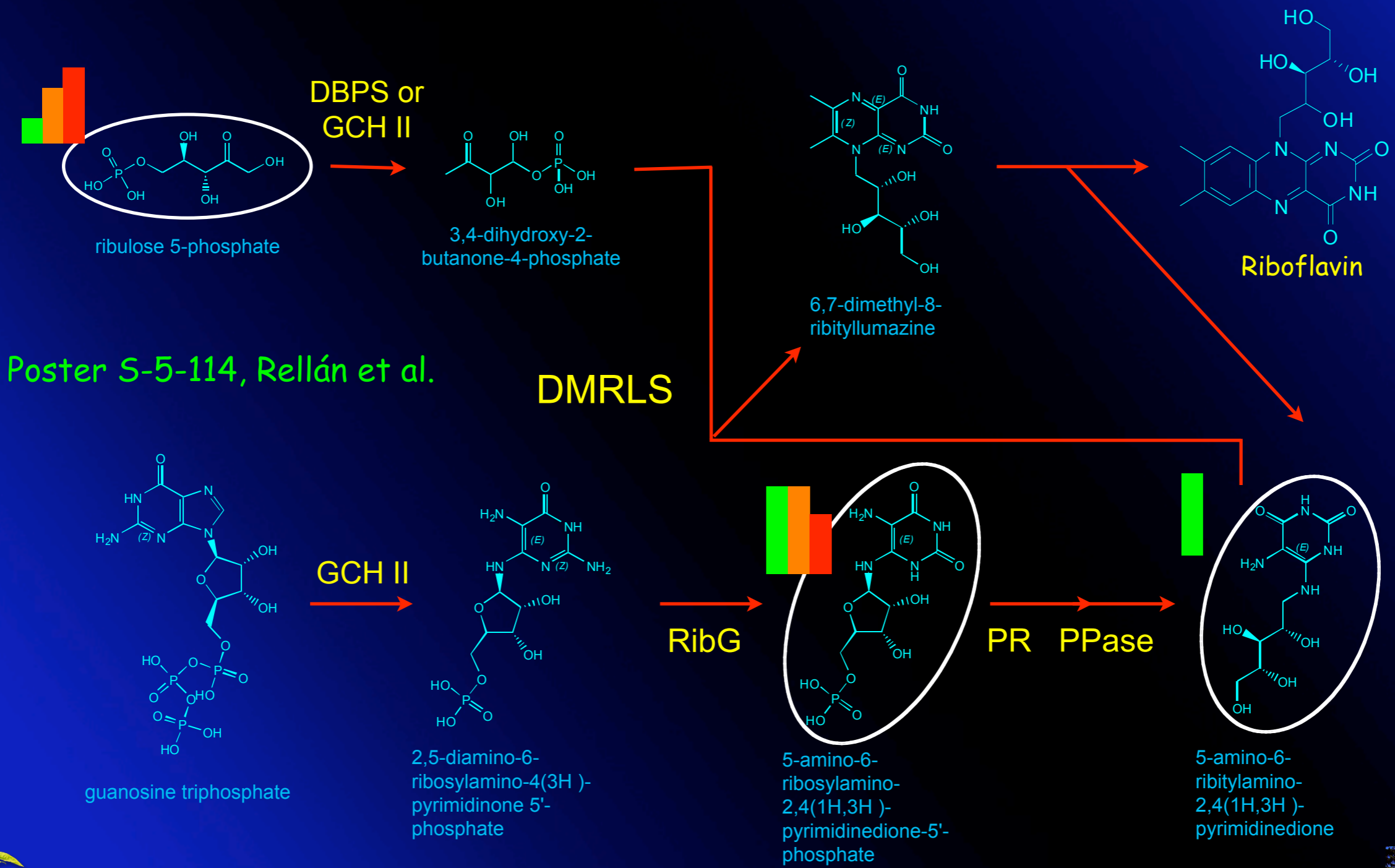


- Fe, pH 8.0



+ Fe  
 - Fe, pH 5.5  
 - Fe, pH 8.0

Riboflavin biosynthesis in plants



Poster S-5-114, Rellán et al.

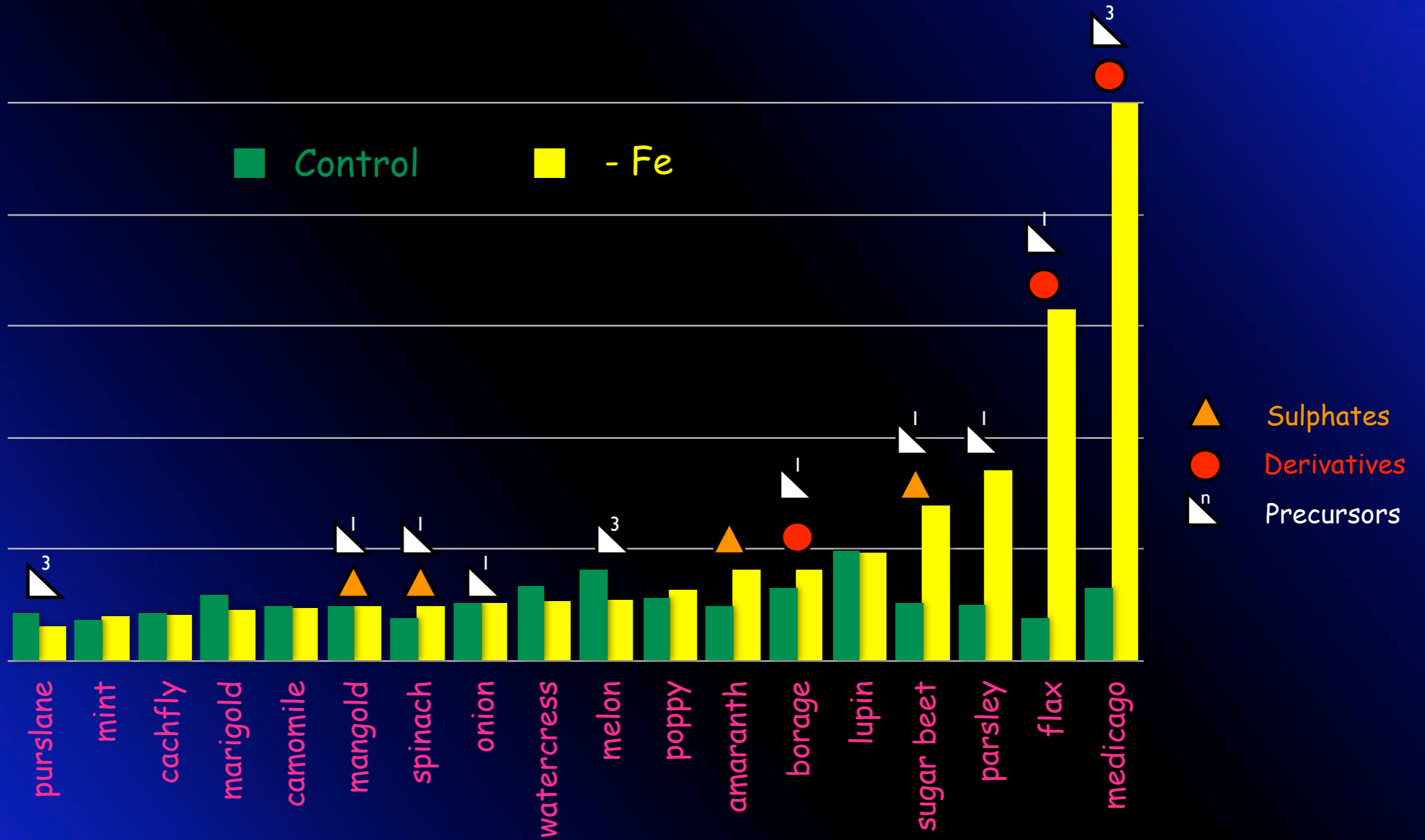
## Current knowledge

- Flavins depend on plant species



● Flavins depend on plant species

riboflavin,  
relative  
units

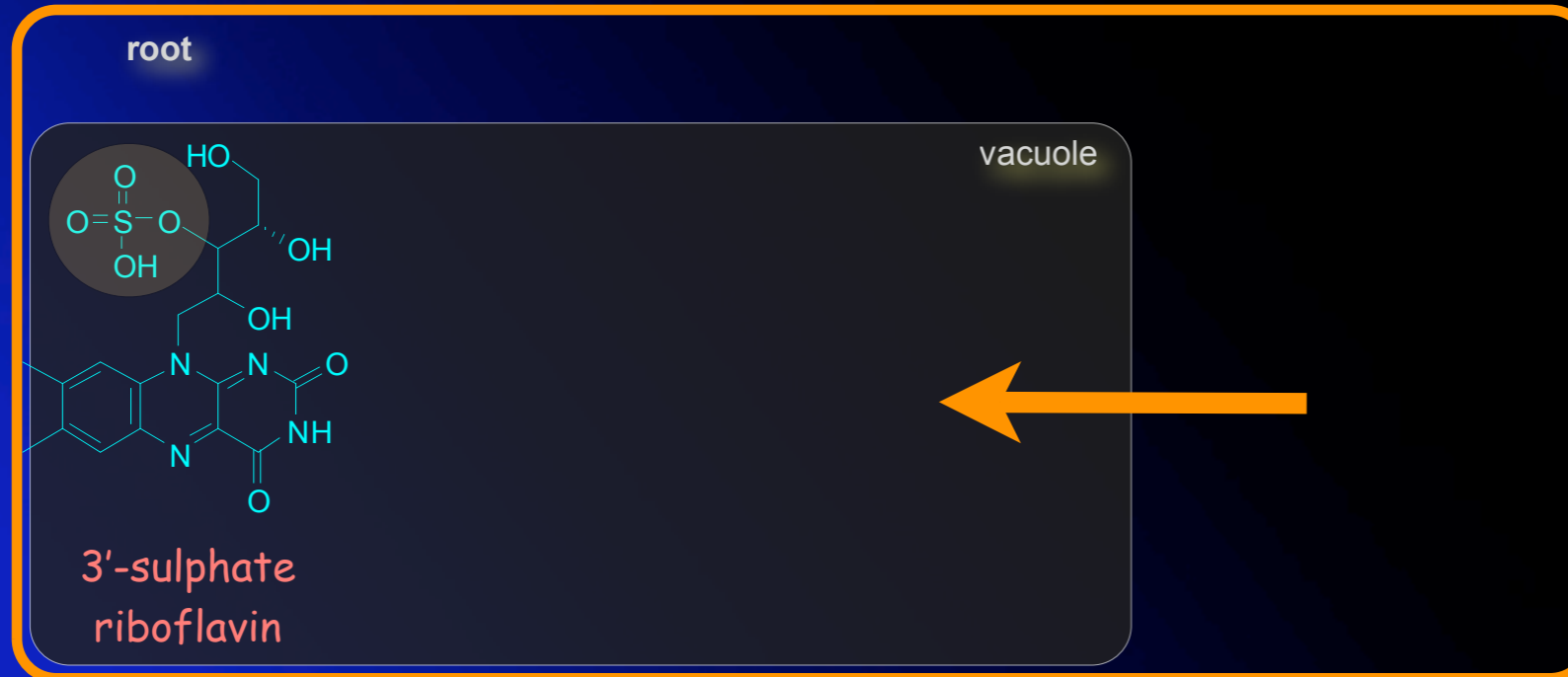
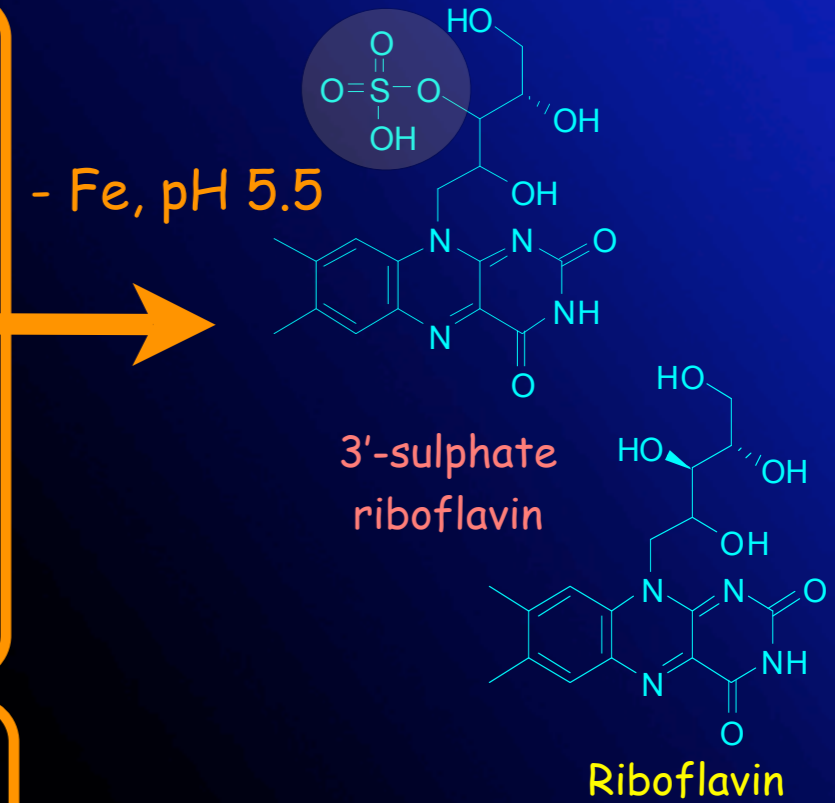
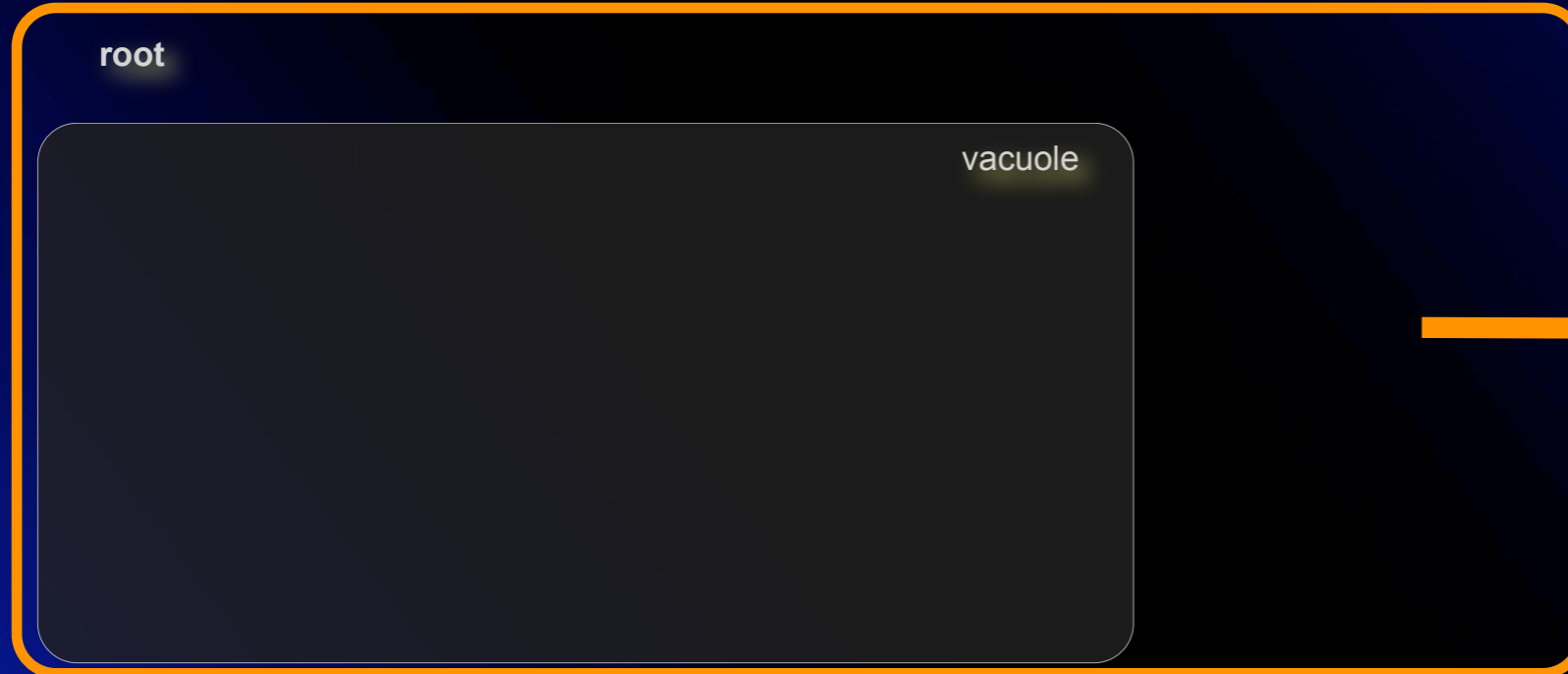


## Current knowledge

- Flavins depend on plant species
- Flavin excretion and accumulation depends on pH



- Flavin excretion and accumulation depends on pH  
Sugar beet

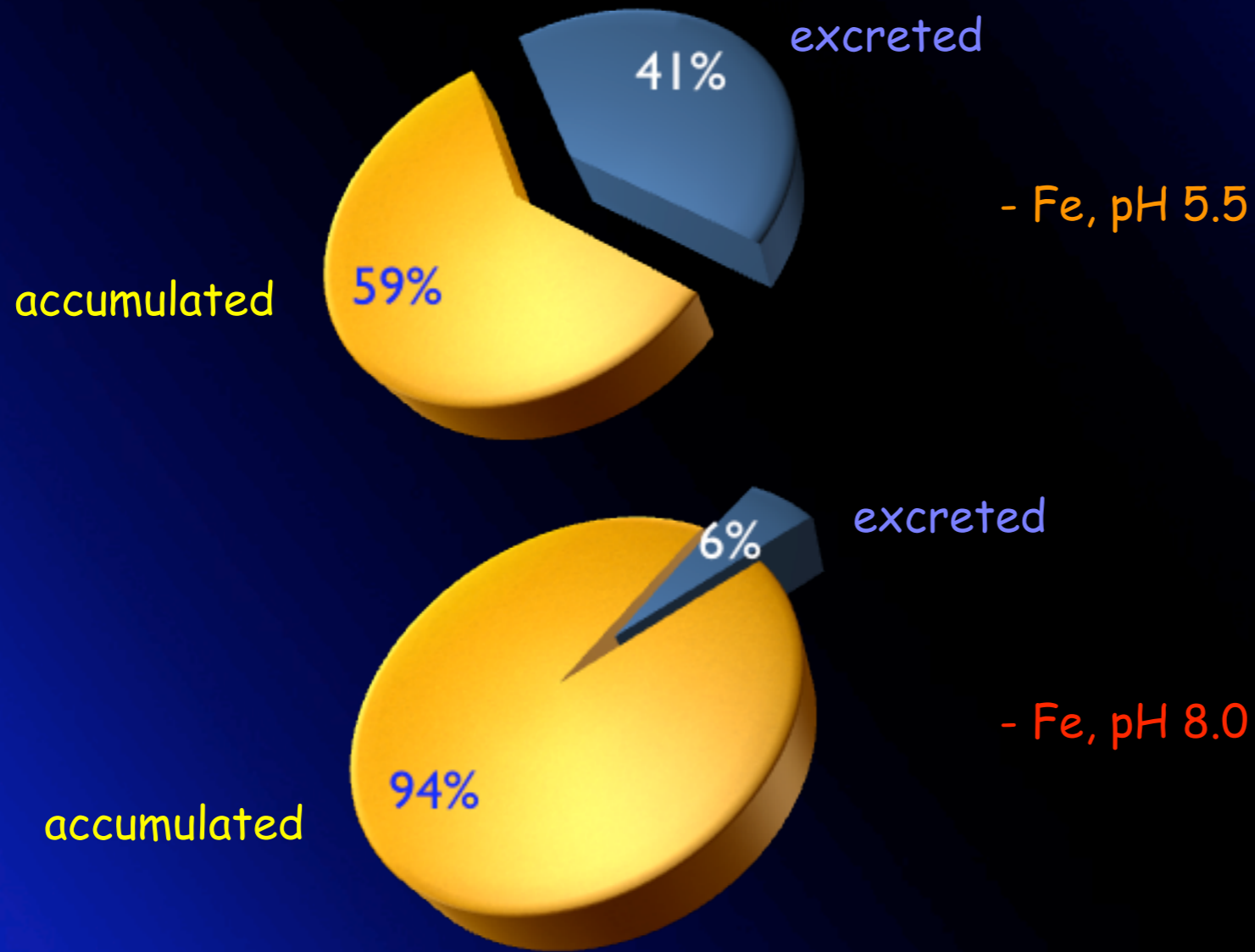


**- Fe, pH 8.0**





- Flavin excretion and accumulation depends on pH  
Medicago truncatula



## Current knowledge

- Flavins depend on plant species
- Flavin excretion and accumulation depends on pH
- Transporters (plasma membrane, vacuole)



- Transporters (plasma membrane, vacuole)  
Sugar beet



## Possible roles

- Cytosolic electron transport bridge
- Antimicrobial agent in the rhizosphere
- Extracellular electron transport bridge?

2007 **PNAS**

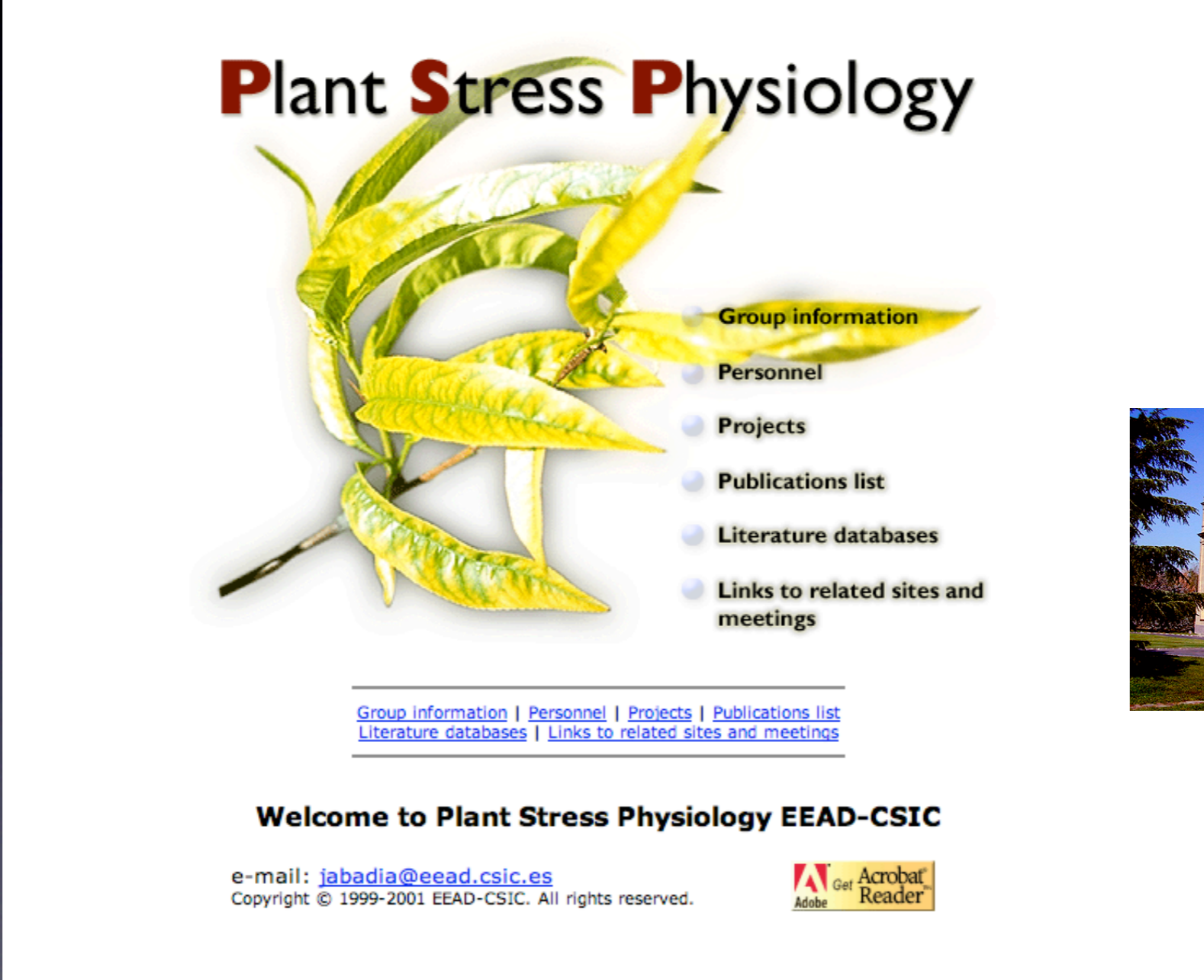
***Shewanella* secretes flavins that mediate extracellular electron transfer**

Enrico Marsili<sup>\*</sup>, Daniel B. Baron<sup>\*</sup>, Indraneel D. Shikhare<sup>\*</sup>, Dan Coursolle<sup>\*</sup>, Jeffrey A. Gralnick<sup>\*,†</sup>, and Daniel R. Bond<sup>\*,†,‡</sup>

5 Staff: Javier Abadía, Anunciación Abadía, Fermín Morales, Ana-Flor López-Millán, Ana Álvarez-Fernández  
1 Postdoc: Saúl Vázquez

6 Predoc Fellows: I. Orera, R. Rellán, R. Sagardoy, J. Rodríguez, H. El Jendoubi, G. Lattanzio

3 Technicians: Ade Calviño, Aurora Poc, Víctor del Río




# Plant Stress Physiology

- Group information
- Personnel
- Projects
- Publications list
- Literature databases
- Links to related sites and meetings

[Group information](#) | [Personnel](#) | [Projects](#) | [Publications list](#)  
[Literature databases](#) | [Links to related sites and meetings](#)

**Welcome to Plant Stress Physiology EEAD-CSIC**

e-mail: [jabadia@eead.csic.es](mailto:jabadia@eead.csic.es)  
Copyright © 1999-2001 EEAD-CSIC. All rights reserved.

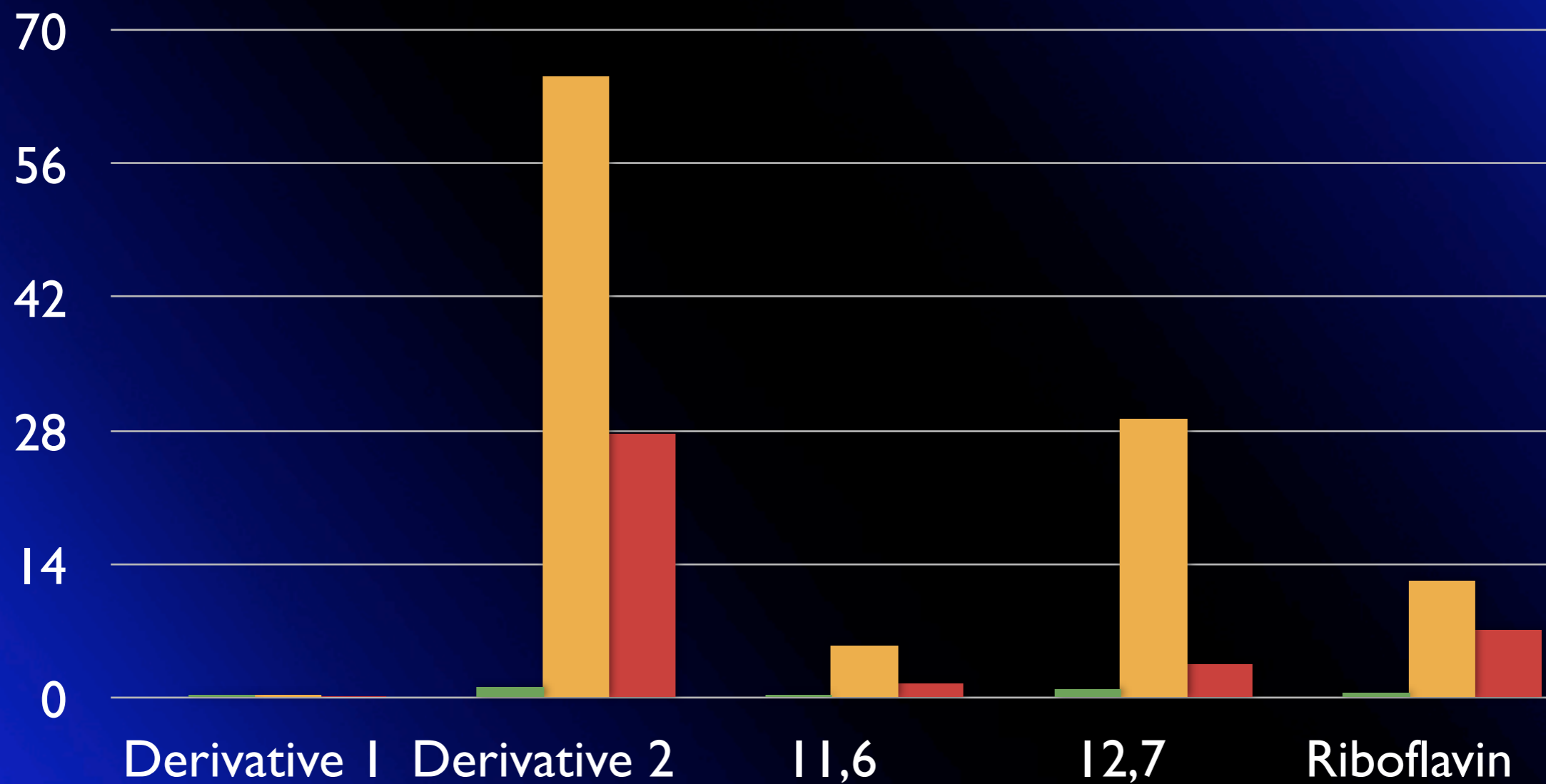


[www.eead.csic.es/stressphysiology](http://www.eead.csic.es/stressphysiology)





Graficas de la cuantificación de  
riboflavina y derivados en los  
concentrados de solución nutritiva

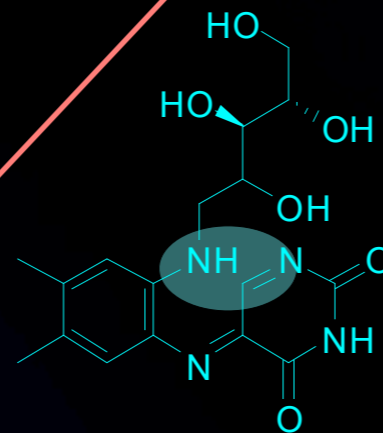


# Medicago truncatula flavin excretion to the solution

Flavins in Fe-deficient *Medicago truncatula*



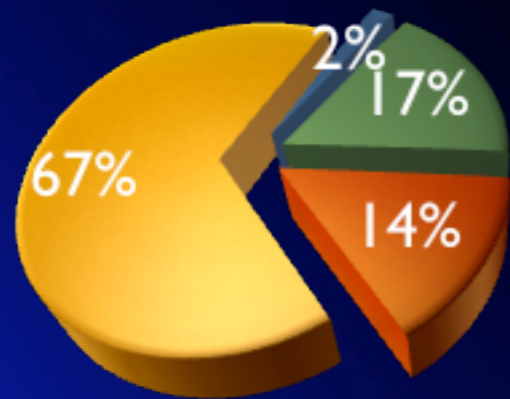
7-alpha hidroxy riboflavin



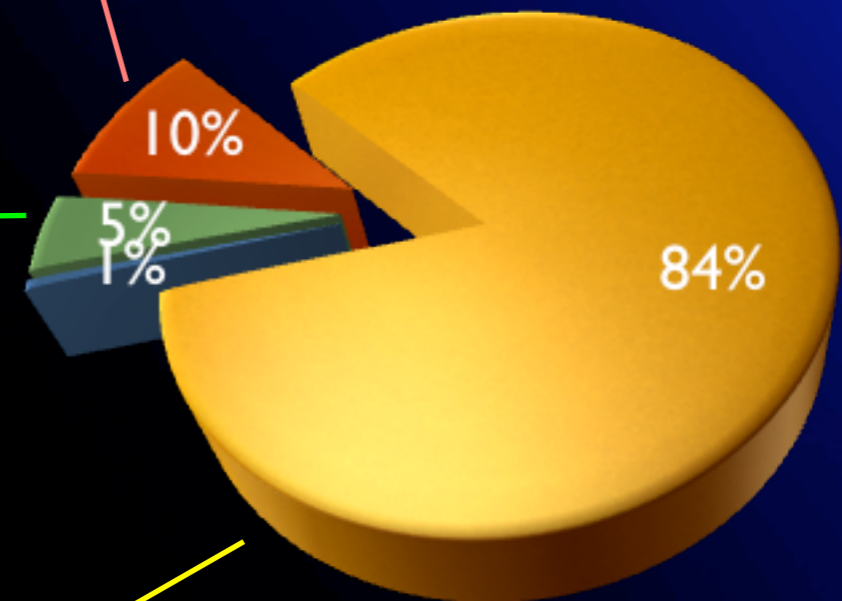
(E)-5-(4,5-dimethyl-2-((3R,4S)-2,3,4,5-tetrahydroxypentylamino)phenylimino) pyrimidine-2,4(3H,5H)-dione



Riboflavin



- Fe, pH 5.5



- Fe, pH 8.0



Planta (2007) 226:147–158  
DOI 10.1007/s00425-006-0476-9

ORIGINAL ARTICLE

## Iron assimilation and transcription factor controlled synthesis of riboflavin in plants

A. Vorwieger · C. Gryczka · A. Czihal · D. Douchkov ·  
J. Tiedemann · H.-P. Mock · M. Jakoby ·  
B. Weisshaar · I. Saalbach · H. Bäumlein

