



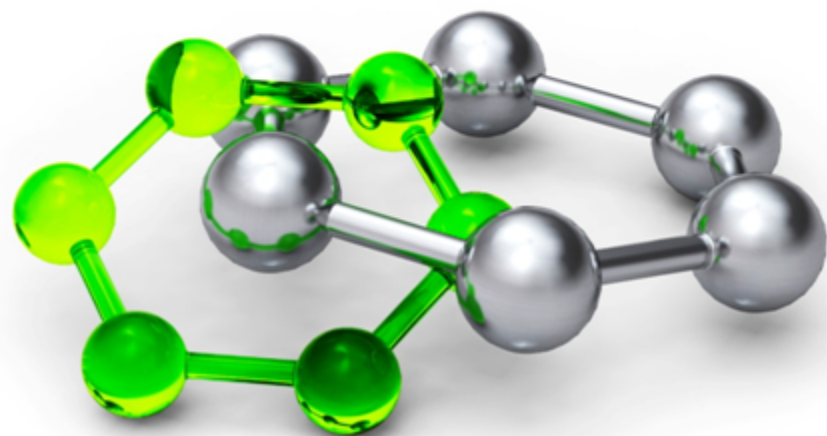
Central European Institute of Technology
BRNO | CZECH REPUBLIC

Vývojová a produkční biologie – omické přístupy

RG-4-10
CEITEC MENDELU

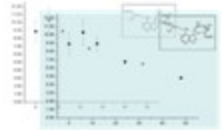


EUROPEAN UNION
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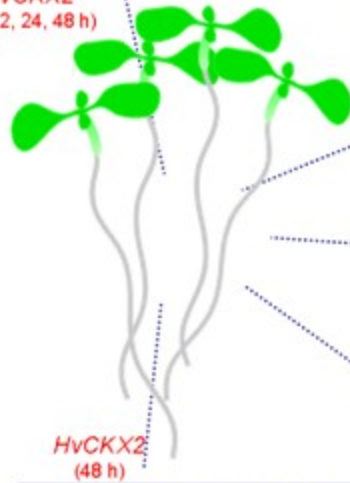
PROTEOMIKA

-Analysis of endogenous cytokinins

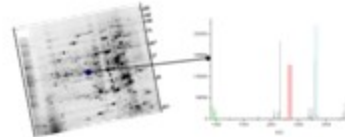


20 analyzed cytokinin metabolites

HvCKX2
(6, 12, 24, 48 h)



-2-DE based analysis



748 detected protein spots

19 differentially regulated

23 identified proteins (4 mixtures)

HvCKX2
(12, 48 h)

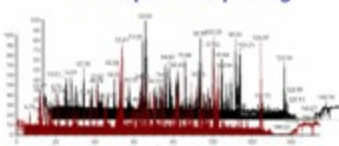
HvCKX2
(48 h)

ipt
(48 h)

HvCKX2
(48 h)

ipt
(48 h)

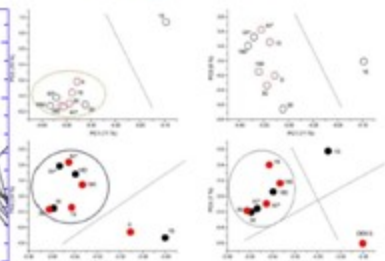
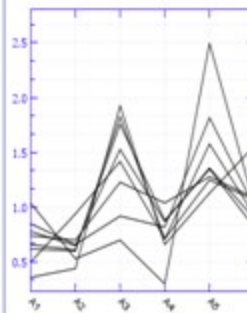
-LC-MS proteomic profiling



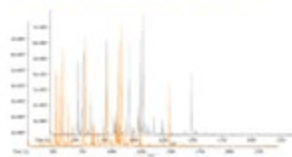
1115 detected proteins

107 differentially regulated (*HvCKX2*)

81 differentially regulated (*ipt*)



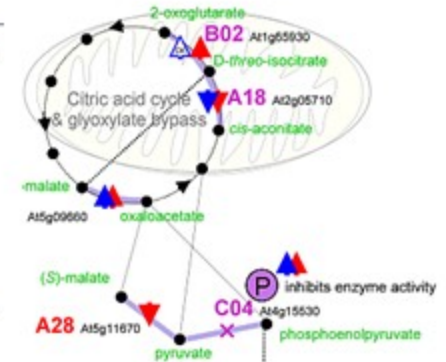
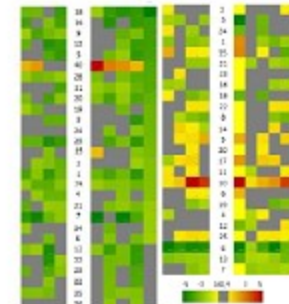
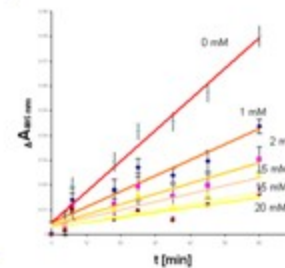
-GC-MS metabolomic profiling



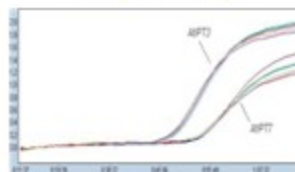
79 detected metabolites

16 differentially regulated (*HvCKX2*)

21 differentially regulated (*ipt*)



-Transcriptomic analysis



11 enzymes involved in cytokinin metabolism, cytokinin reporter

6 differentially regulated

Naše nová laboratoř pro LC-MS



amaZon speed ETD ion trap



- Detection of PTM (including structure of glycosylations)
- High sensitivity (fM, aM)

Nanomate



- Protein-ligand interaction
- LESA



maXis impact UHR-TOF



- Protein ID
- Proteome profiling
- Relative and absolute protein quantification
- Metabolome profiling

Mgr. Jan Skalák

- biofyzika
- proteomika
- fluorescenční techniky

prof. RNDr. Břetislav Brzobohatý, CSc.
vedoucí ústavu



Dr. Martin Černý

- biochemie
- proteomika
- LC-MS

Mgr. Hana Černá

- biochemie
- proteomika
- příprava rekombinantních proteinů



- **extrakce proteinu**

- frakcionace, obohacování proteomu, sledování post-translačních modifikací

- **proteomická analýza**

- elektroforéza (1-DE, 2-DE), LC-MS profilování, relativní/absolutní kvantifikace proteinu pomocí MS, bioinformatika

- **příprava a purifikace rekombinantních proteinů**

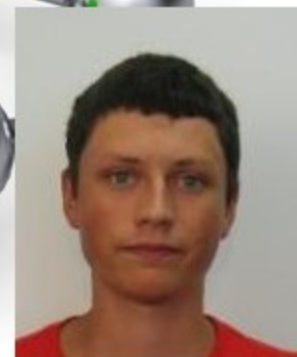
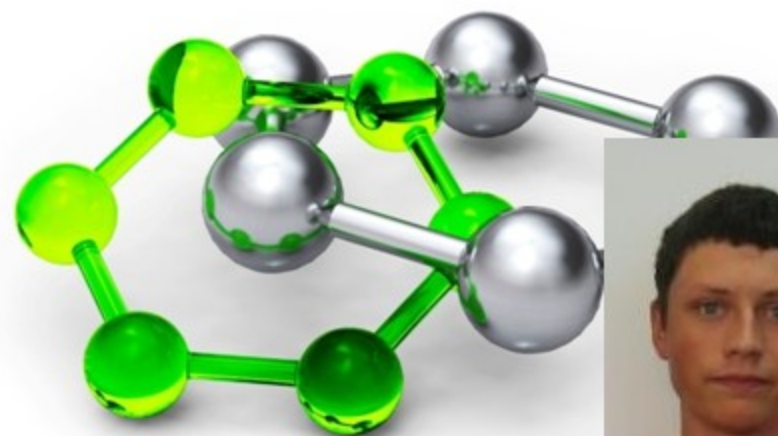
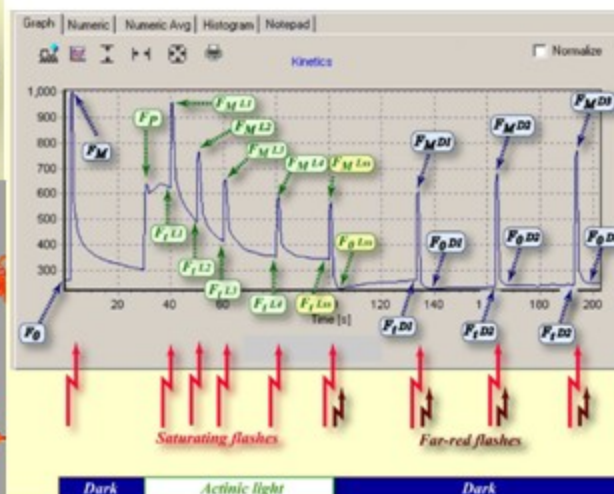
- klonování, optimalizace exprese, purifikace proteinu

- **verifikace proteomických výsledků**

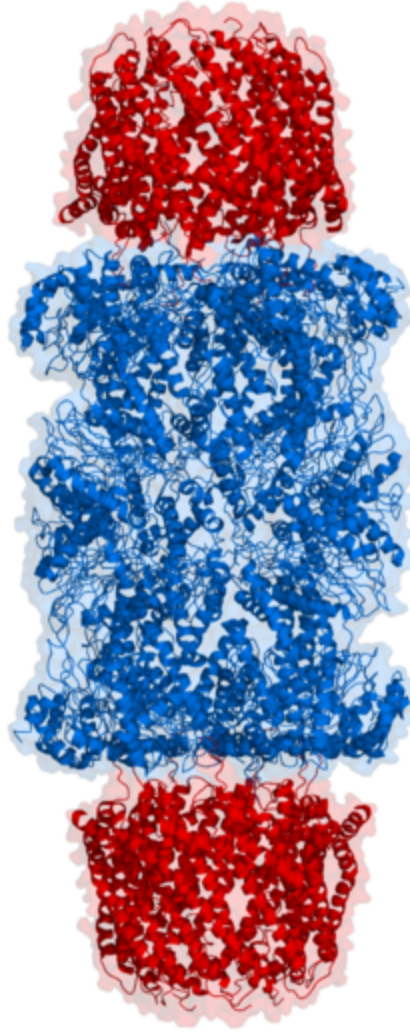
- mikroskopie, RT-qPCR, fyziologické experimenty, fluorescenční analýza,...

Fluorescenční analýza kinetiky chlorofylu rostlin se změněnými hladinami hormonů

Mgr. JAN SKALÁK



Proteasom a jeho role v signalizaci rostlinných hormonů

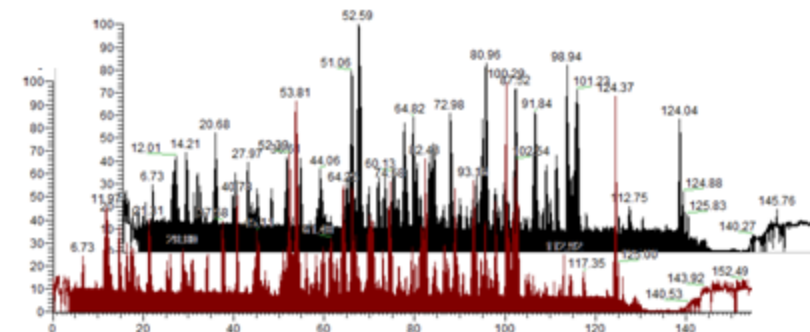
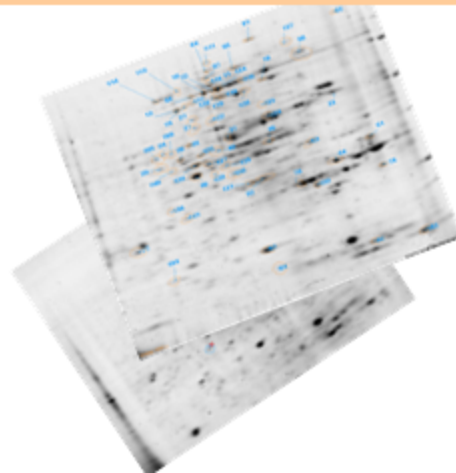


Mgr. Martin Černý, Ph.D.

- purifikace proteinu
- 1-DE, 2-DE, LC-MS
- proteomická analýza

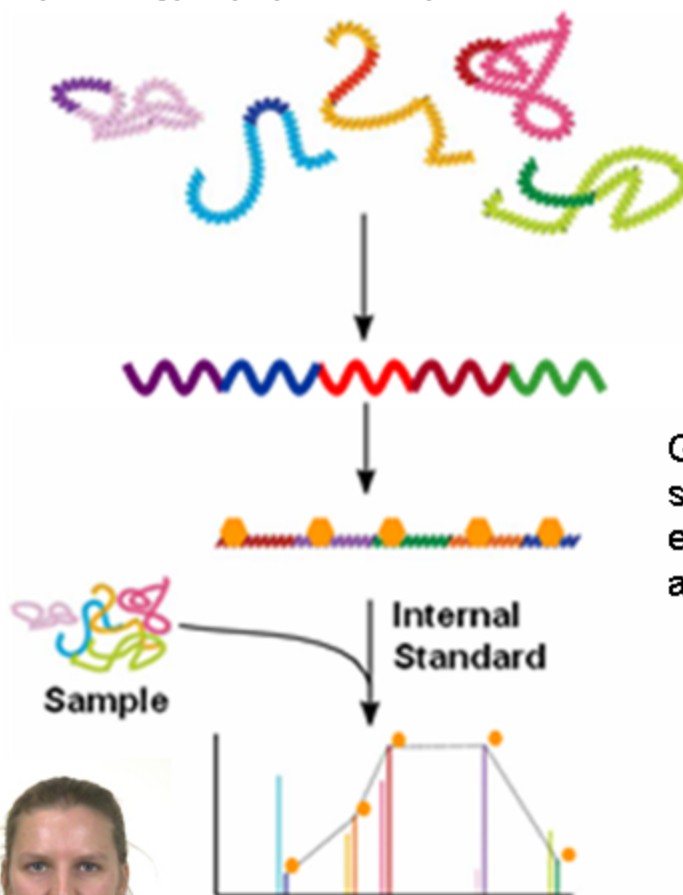
Why?

- Proteasome-mediated signaling is crucial for IAA, JA or GA signaling, but seems to be important for all known plant hormones (e.g. Sadanandom et al., 2012)



Relativní a absolutní kvantifikace proteinů pomocí hmotnostní spektrometrie

Synthetic gene is designed to encode
proteotypic peptides of proteins of interest



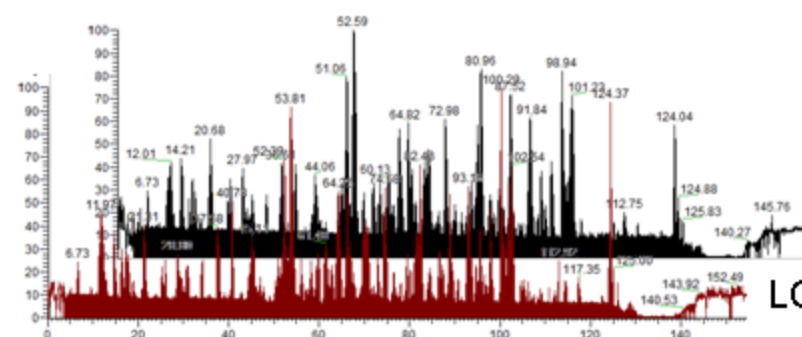
Mgr. Hana Černá

- Příprava rekombinantních proteinů
- Purifikace proteinu
- 1-DE

Mgr. Martin Černý, Ph.D.

- LC-MS

Gene is synthesized,
subcloned into vector and
expressed (isotope labeled
amino acids)



LC-MS analysis

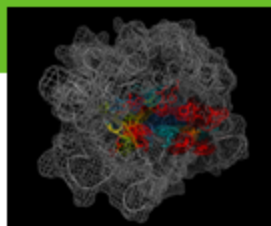


For those who are interested in biodesign

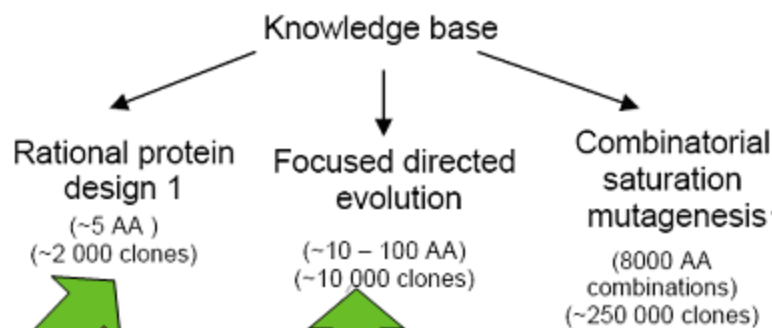
•**Proteinové inženýrství pro výzkum hormonálního systému rostlin**



Mgr. Pavel Mazura, Ph.D.
mazura@sci.muni.cz



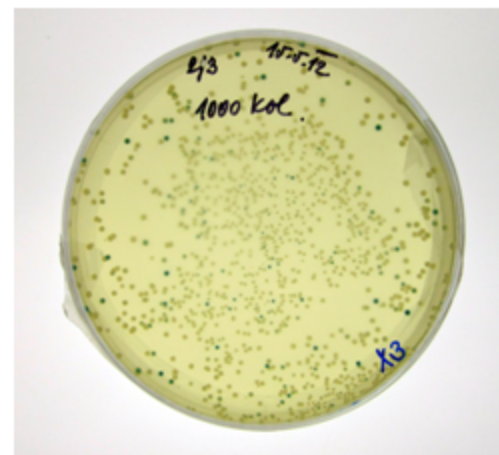
New mutagenesis design



W373X saturation mutagenesis



Enzyme kinetics and screening based on glucose detection

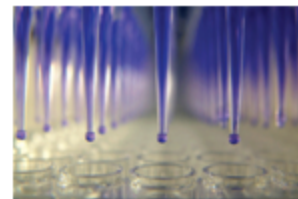
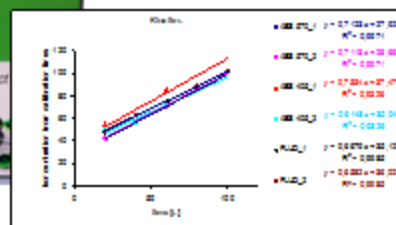
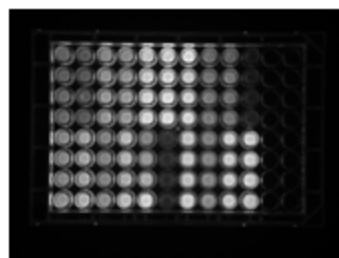


7792 clones 15-20% positive
→ 50 000 positive clones

(cca 1500 unique functional β -glucosidases out of 8000)

8000 clones technology

1. powerful screening method
2. HT mutation identification method
3. cutting edge equipment (CEITEC)



- témata jsou/budou nabízena prostřednictvím IS MU
- kontakt na jednotlivé školitele:

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Scaled Biosystems Pavel Mazura - mazura@sci.muni.cz