Role of ERD14 as a plant chaperone:

A Structural Biology approach.

Cesyen Cedeño
VIB - Brussels

OUTLINE

Review of current topic of research

Own Approach: my experience dealing with ERD14

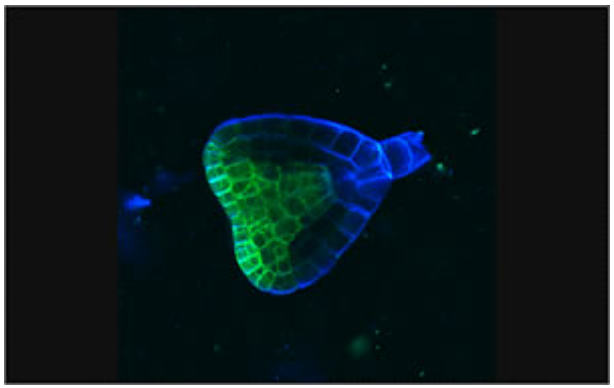
Progress made:

- Structure calculation
- Delivering proteins inside cells
- NMR data collection

Review…

LEA – Late Embryogenesis Abundant Proteins

Their precise function is unknown, but they are assumed to protect cellular or molecular structures from the damaging effects of water loss… (reported from cotton seed's development studies*)

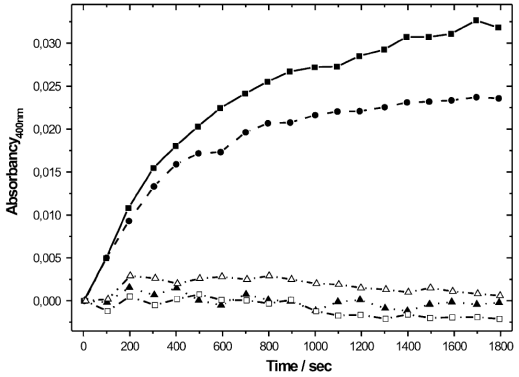


ERD14 – ERD10 are Lysine rich or KS_3 (classification)

K segment: EKKGIMDKIKEKLPG
S segment: SDSSSSSSSEEEGS (ERD14)

Chaperoning (Plant Physiology, May 2008, Vol. 147, pp. 381–390)

Antiaggregation: using Luciferase as a probe for heating induced aggregation ERD14/10 has similar effect to that of Hsp90.



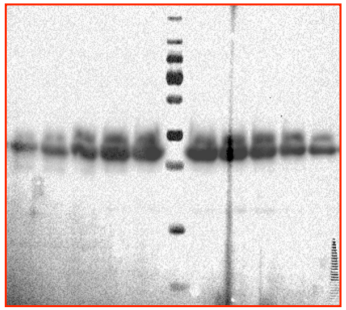
Own approach…

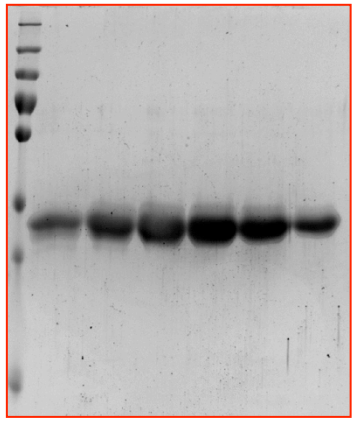
Once upon a time…

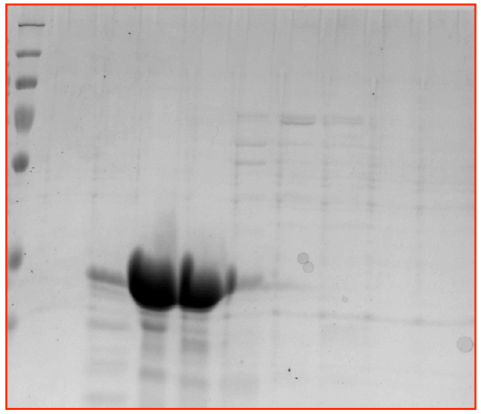
ERD14 purification

Intrinsically Disordered Proteins can be boiled… and they survive!!!

Binding to IEX column + GF







However:

We set up OUR lab from scratch and is still “under construction”

Including a plant biology section for *in planta/in vivo* experimentation.

OUTLINE

Review of current topic of research

Own Approach: my experience dealing with ERD14

Progress made:

- Structure calculation
- Delivering proteins inside cells
- NMR data collection

Progress…

STRUCTURE CALCULATION from NMR data:
M. Vendruscolo's // M Blackledge approach
Can PREs be collected *in cell*?

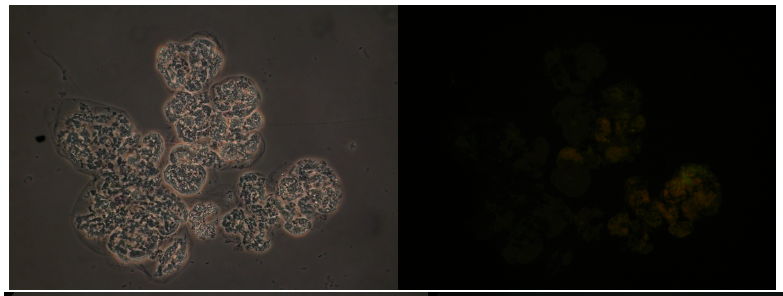
Near future

ELECTROPORATION:
Delivering a model protein with a fluorescent probe (BSA-FITC)

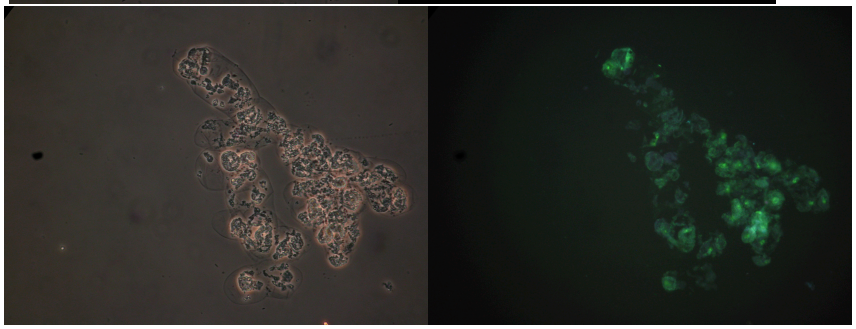
- Use of a fluorescent probe attached to ERD14 to confirm delivery inside cells

OVEREXPRESSION:
Get confirmation of an ERD14 inducible system *in planta*
Will require optimization to get labeling conditions + *in cell* spectra

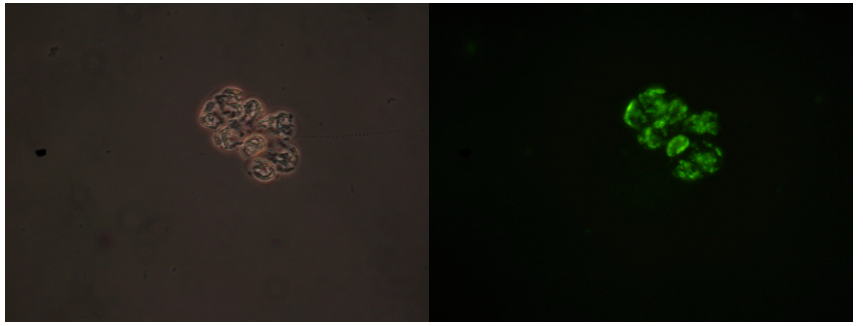
in cell…



Plant cells before treatment without probe

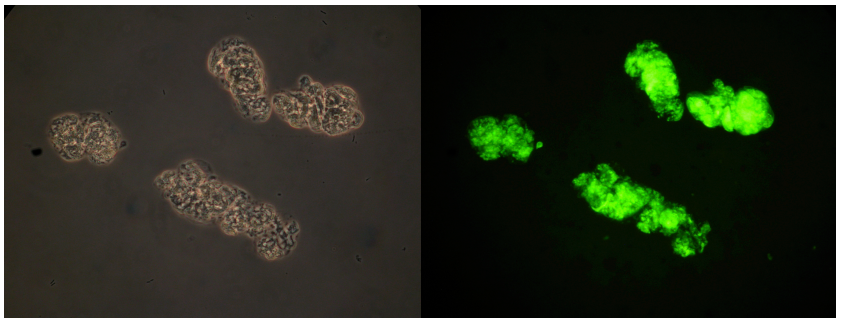


Treated cells (probe added)

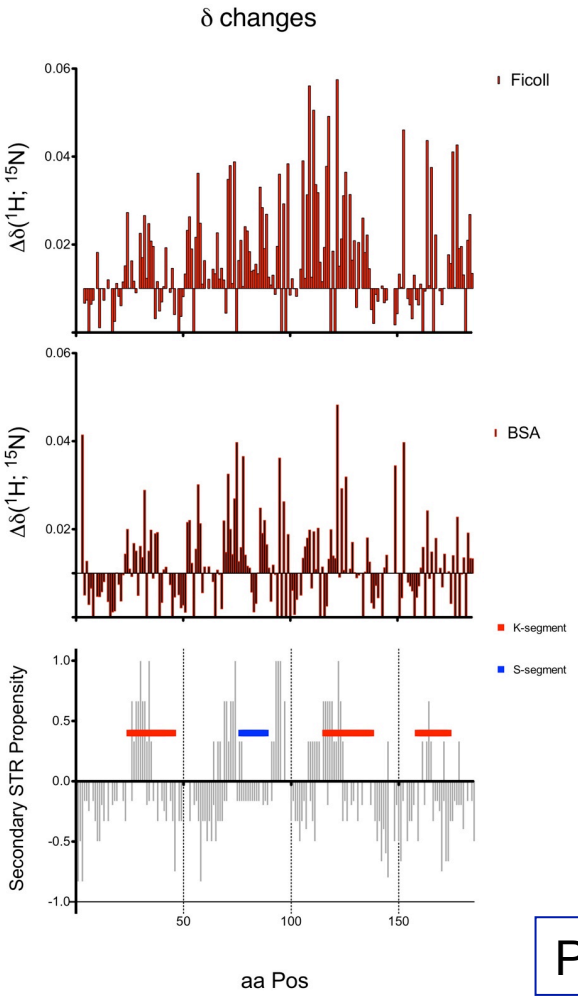


Treated + recovered cells (probe added)

Treated + ELECTROPORATED recovered cells (probe added)



NMR data acquisition…



FMP Berlin:
Phillip Selenko & Andres Binolfi

Testing crowding conditions before getting the protein inside

However phosphorylation of S segment by CKII was studied both *in vitro* (based on published data)
[Calcium binding assays, phosphoproteomics]

How does it affects chaperoning?
What is the balance between Kinases/Phosphatase during stress?

Phosphorylation + Structural changes + partner recognition