



Functional dissection of a disordered plant chaperone ERD14

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Structural definition of protein disorder

- Protein / region without well defined tertiary structure (intrinsically *disordered* protein, IDP) in its native form
- Lack of hydrophobic residues
- No hydrophobic core
- In solution, in cell, with and without partner ?????

Laboratory definition of disordered proteins

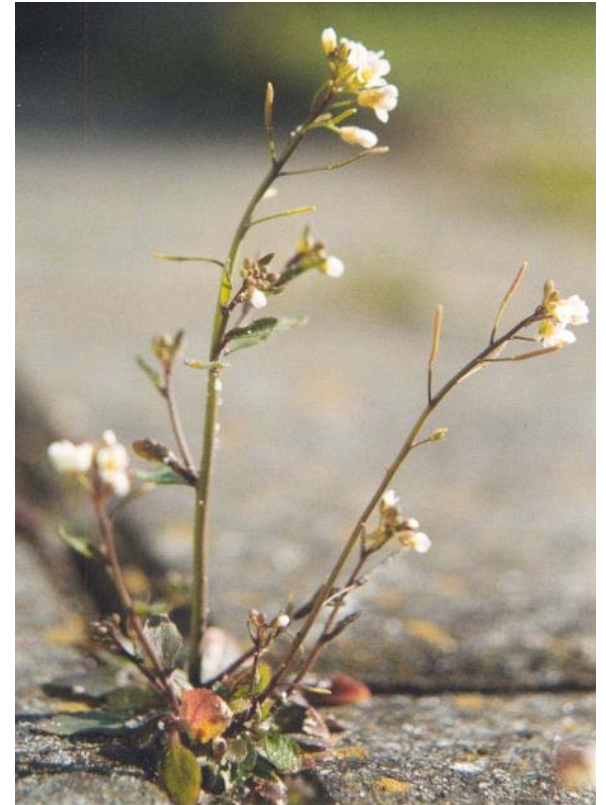
- Non-crystallizable
- Specific biophysical parameters (CD, DSC, NMR)
- Heat stability
- Shifted run on SDS-PAGE
- Sensitivity to limited proteolysis

Functional definition of protein disorder

- Globular / disordered
- No enzymatic activity
- Primary role: interaction partners
- PresMo, MoRE, ELM
- Inhibitor, adaptor, scaffold
- High incidence in regulatory processes (signal transduction, cell cycle, transcriptional regulation)

ERD14 protein

- ERD: Early Response to Dehydration
- *Arabidopsis thaliana*
- Expressed in the seed and actively dividing tissues



LEA proteins, dehydrins

- Late embryogenesis-abundant
- Dehydrin (DHN): high salt, desiccation, high / low temperature, abscisic acid
- DHN:
 - K-segment: 15 aa, Lys rich, common
 - Y-segment: 6aa, rare
 - S-segment: 12-18 aa, Ser rich, rare

ERD14

- 185 AS, 20 kDa
- Conserved motifs:

- K segment ->

EKKGFMEKLKEKLPG

- S segment ->

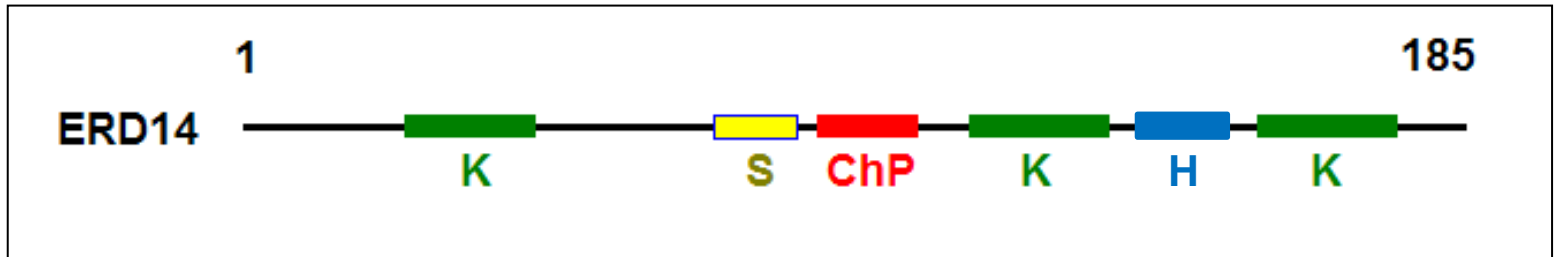
LHRSDSSSSSSS

- ChP segment ->

EEEGSDGEKRKKKKEKKK

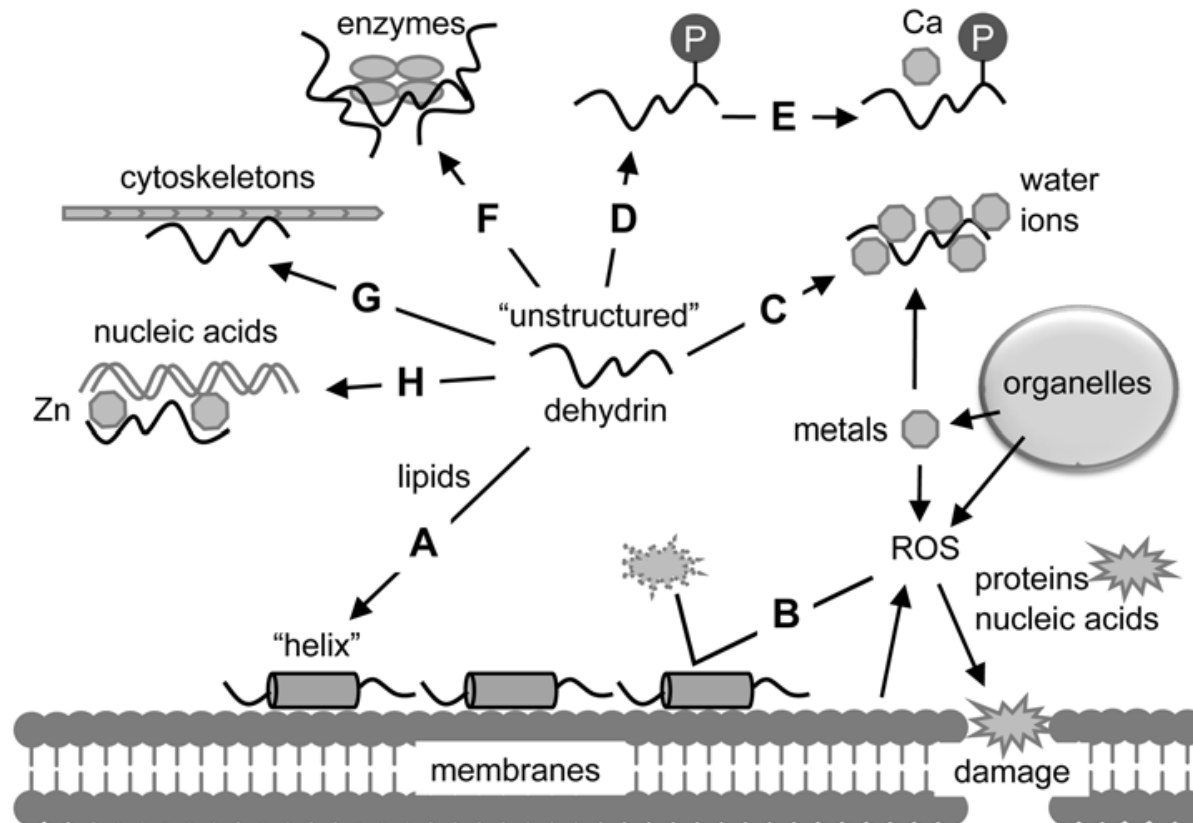
- H segment ->

GSAVAAAPVVVPPP



DHN

- Physiological partners and molecular mechanism **not known**:

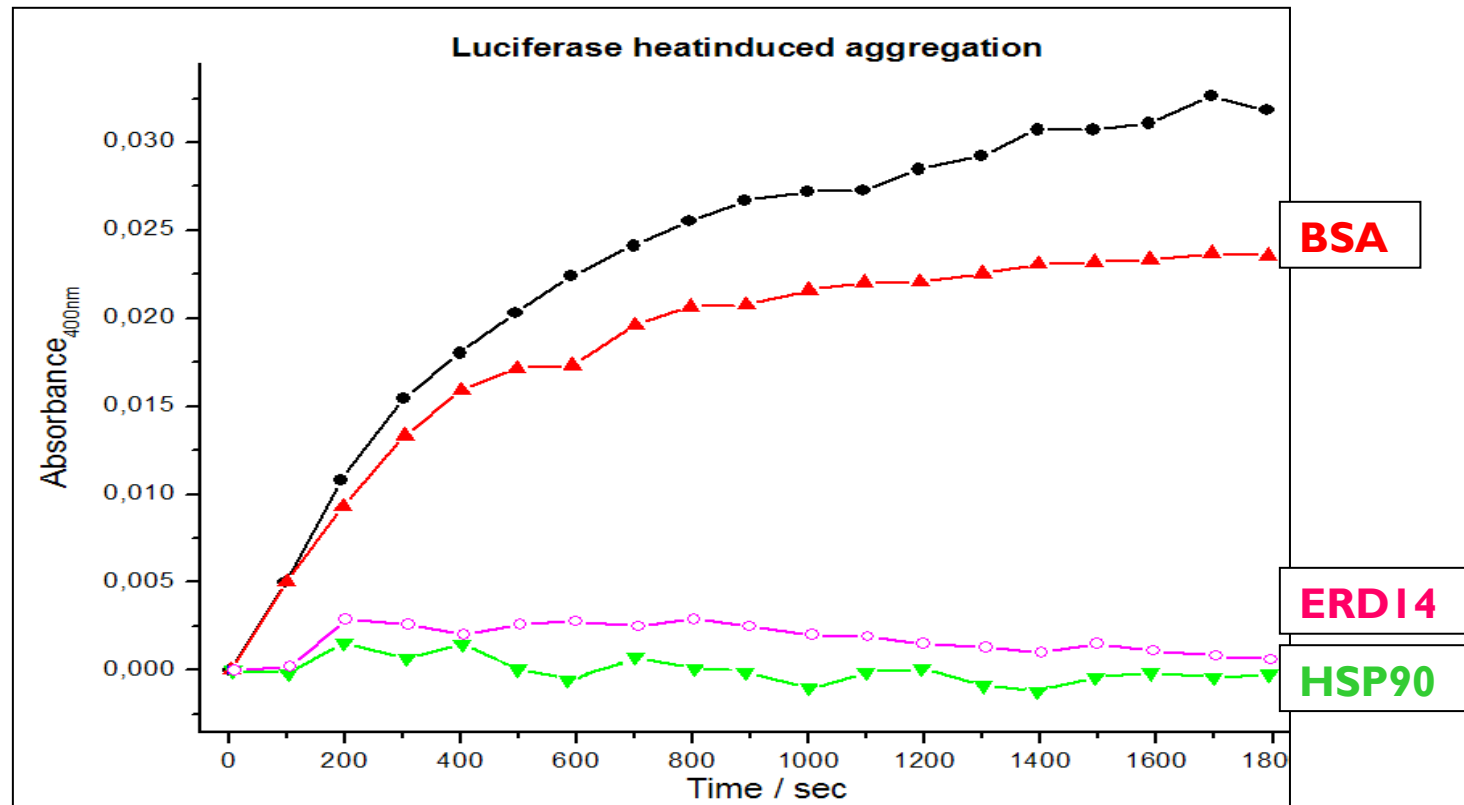


ERD14 in vitro structure

- IDP:
 - CD
 - NMR
 - SDS-PAGE
 - in silico (IUPred, PONDR)
 - Heat stable
 - Protease sensitive

ERD14 in vitro chaperone function

- In vitro chaperone assays
- ADH, lisosyme, citrate-syntase, luciferase



Aims

- Structure in vivo
- Function in vivo
- Functional dissection in vivo

ERD14 in cell / in vivo

- In vivo NMR structure and cell defense against stress in *E. coli*

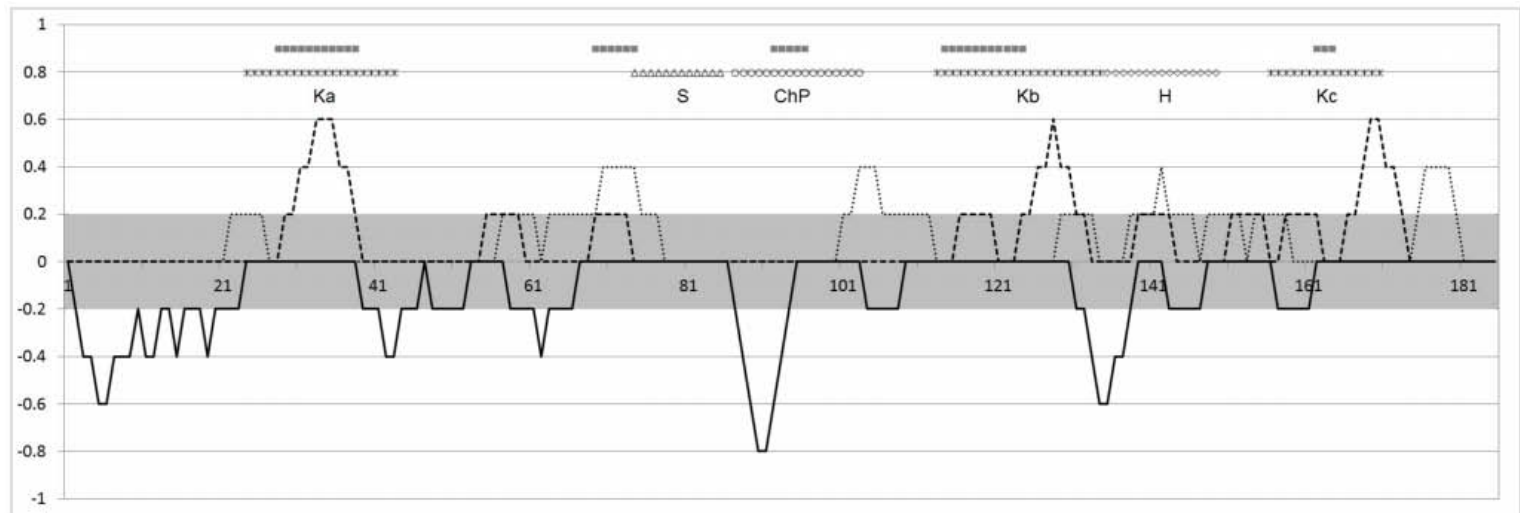
	A. thaliana	E. coli
Physiological partners	+	-
Potential partners	+	+
Cell transformation	?	+
Crowding	+	+
Expression level	?	+
In cell environment	+	+
In cell NMR	?	+
Survival measurements	?	+

In cell spectrum
500 MHz, 277K at pH 7.7
110 / 185 peaks

Supernatant of the in cell experiment

In cell NMR

Disappearance and broadening in the conserved segments

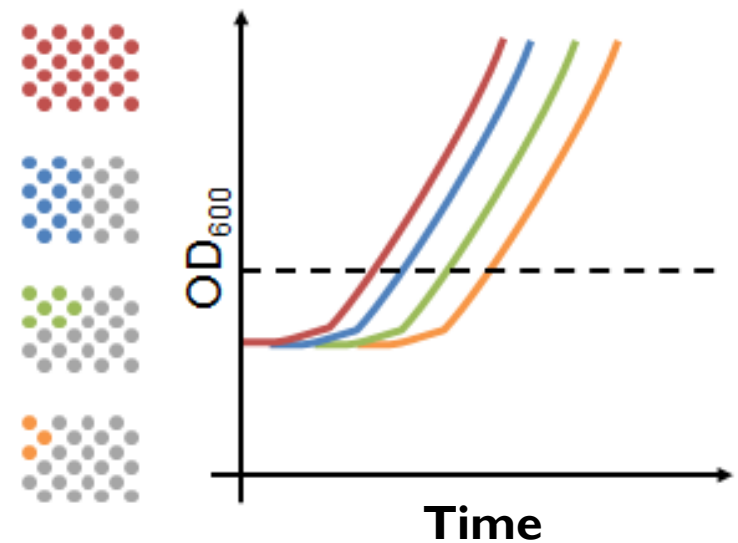
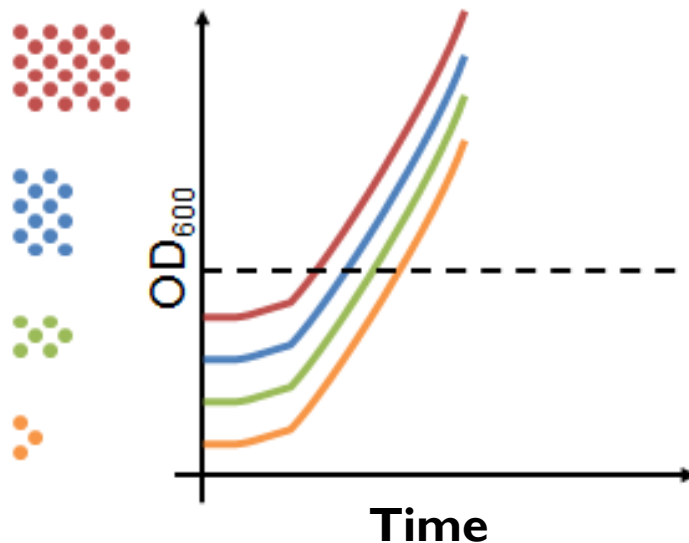


Survival of ERD14 expressing cells after stress

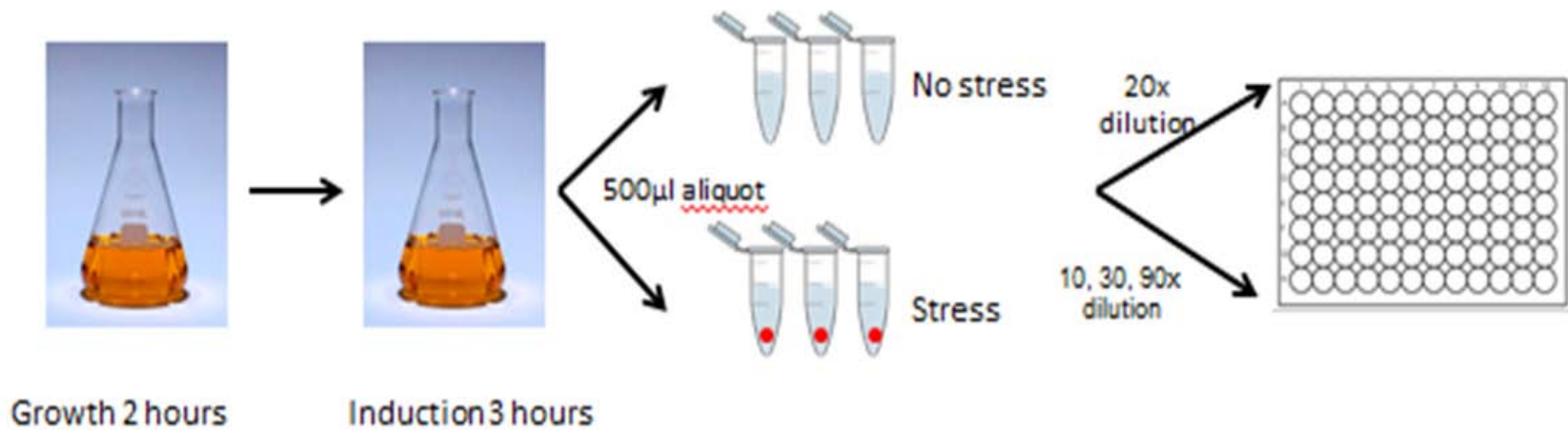
- Fast and reproducible test needed
- BacTiter-Glo™ assay → not reproducible
- Colony counting → high SD, time consuming
- Designing a novel fast and reliable method to measure the survival

Survival measurement method

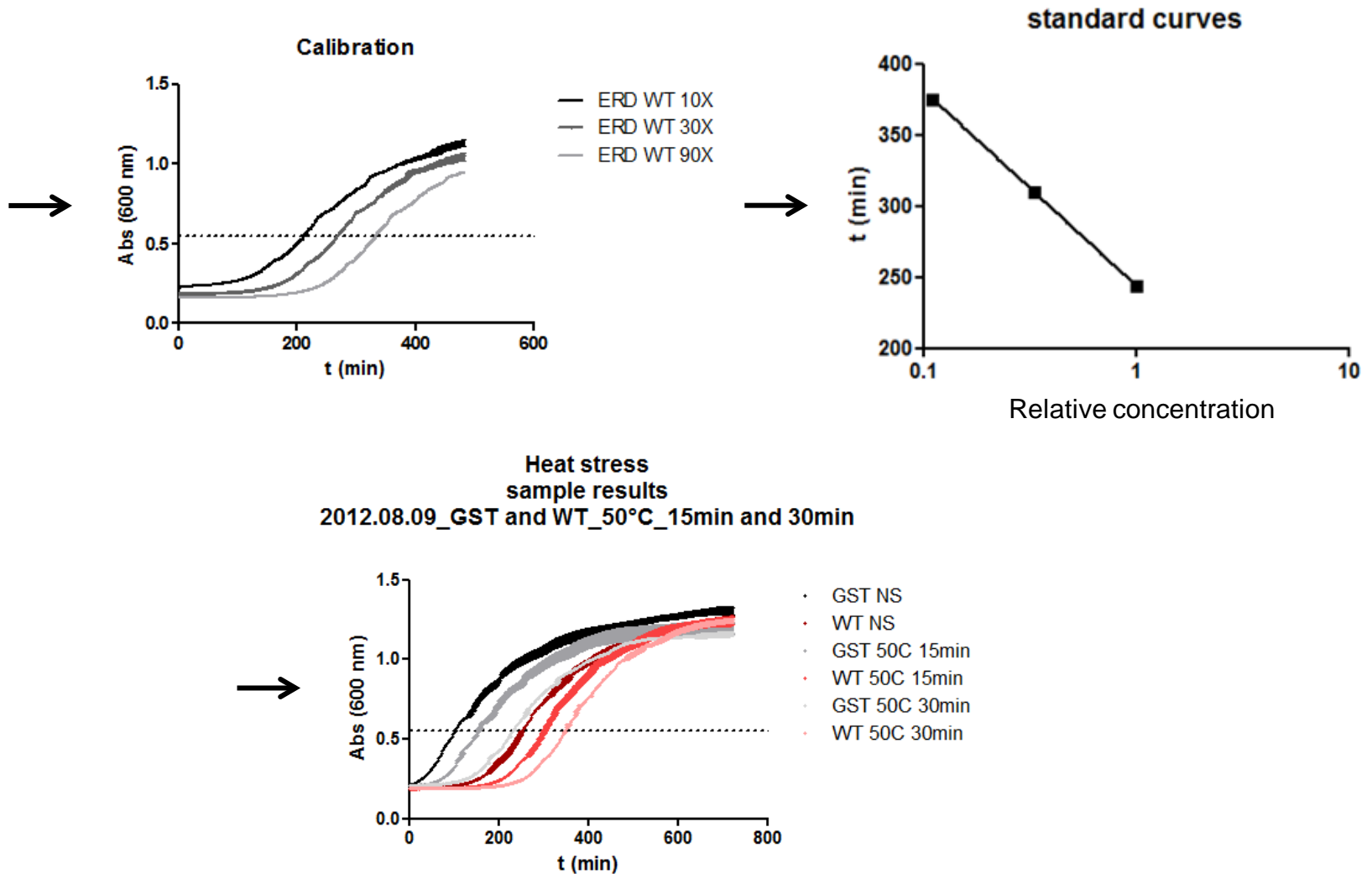
- New method → comparing growth curves, counting relative concentration and survival



Survival measurement method



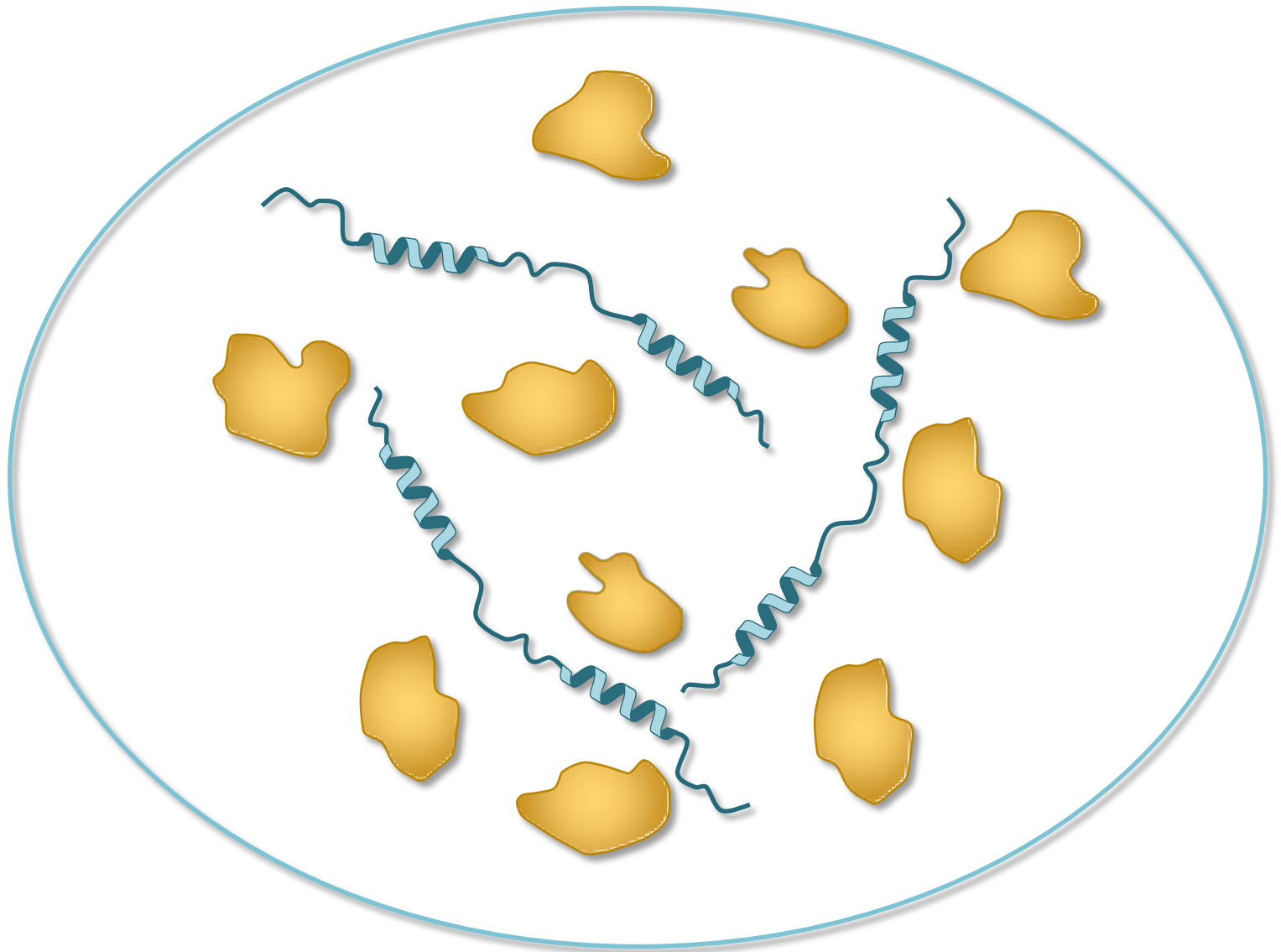
Survival measurement method



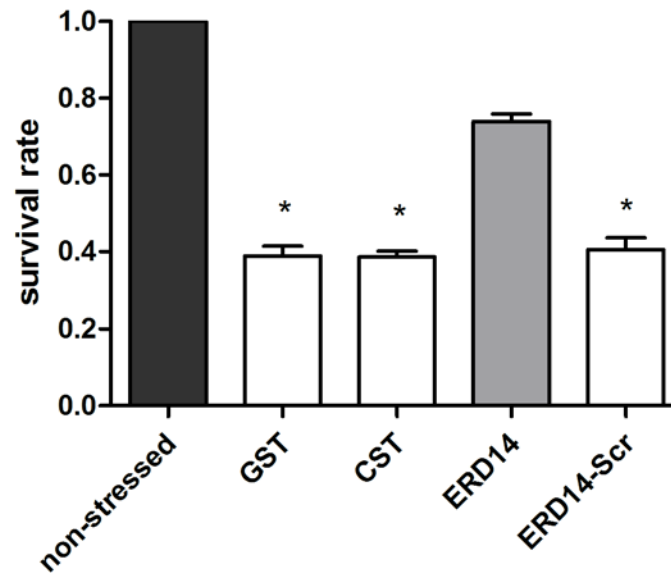
Optimizing stress conditions

- 2M NaCl – affects the growth curve
- Desiccation – not reproducible
- PEG – high SD due to inhomogeneity
- Transient heat stress - 50°C – 15 min

Theory I.: Spacefilling

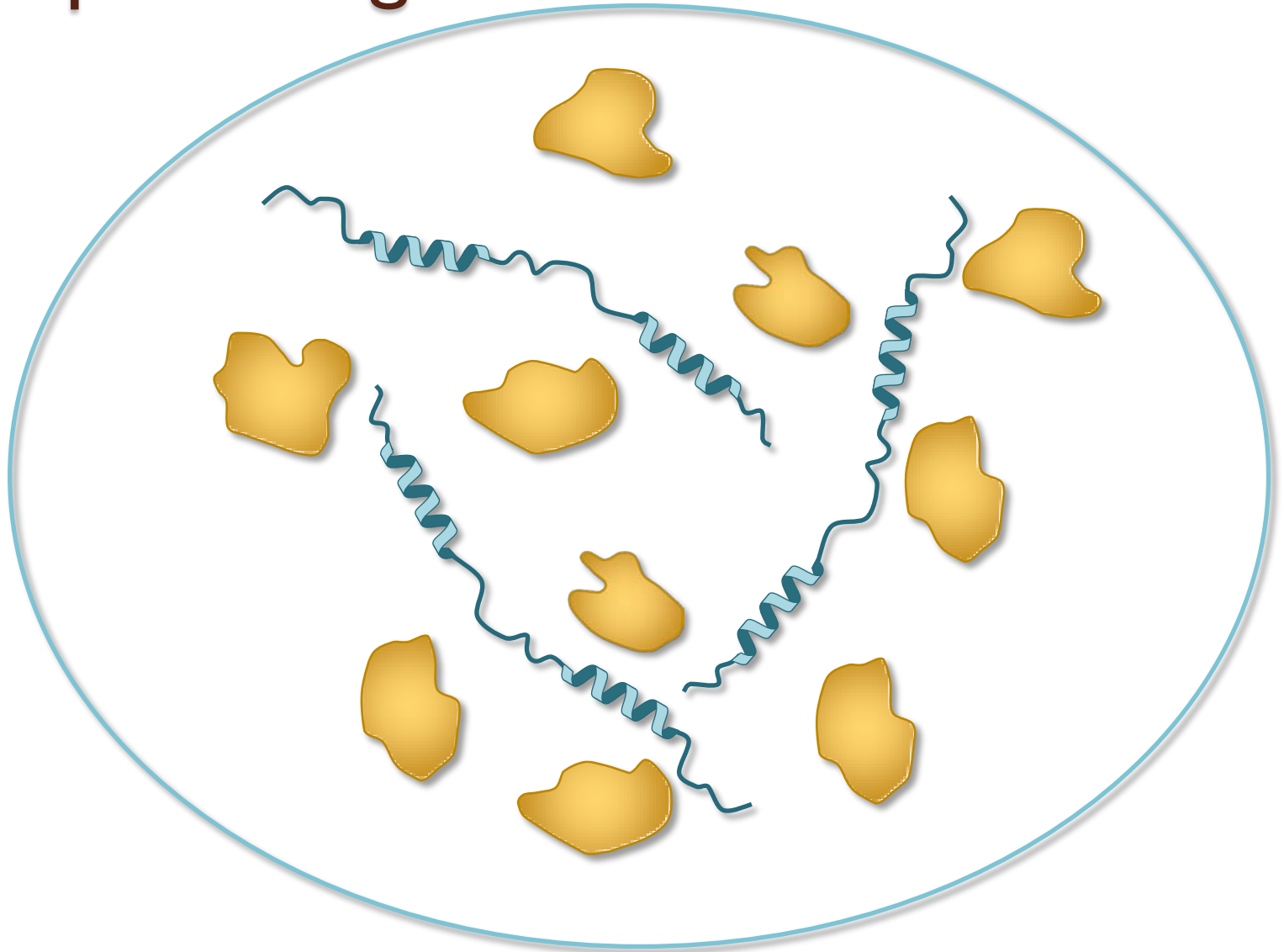


Theory I.: Spacefilling

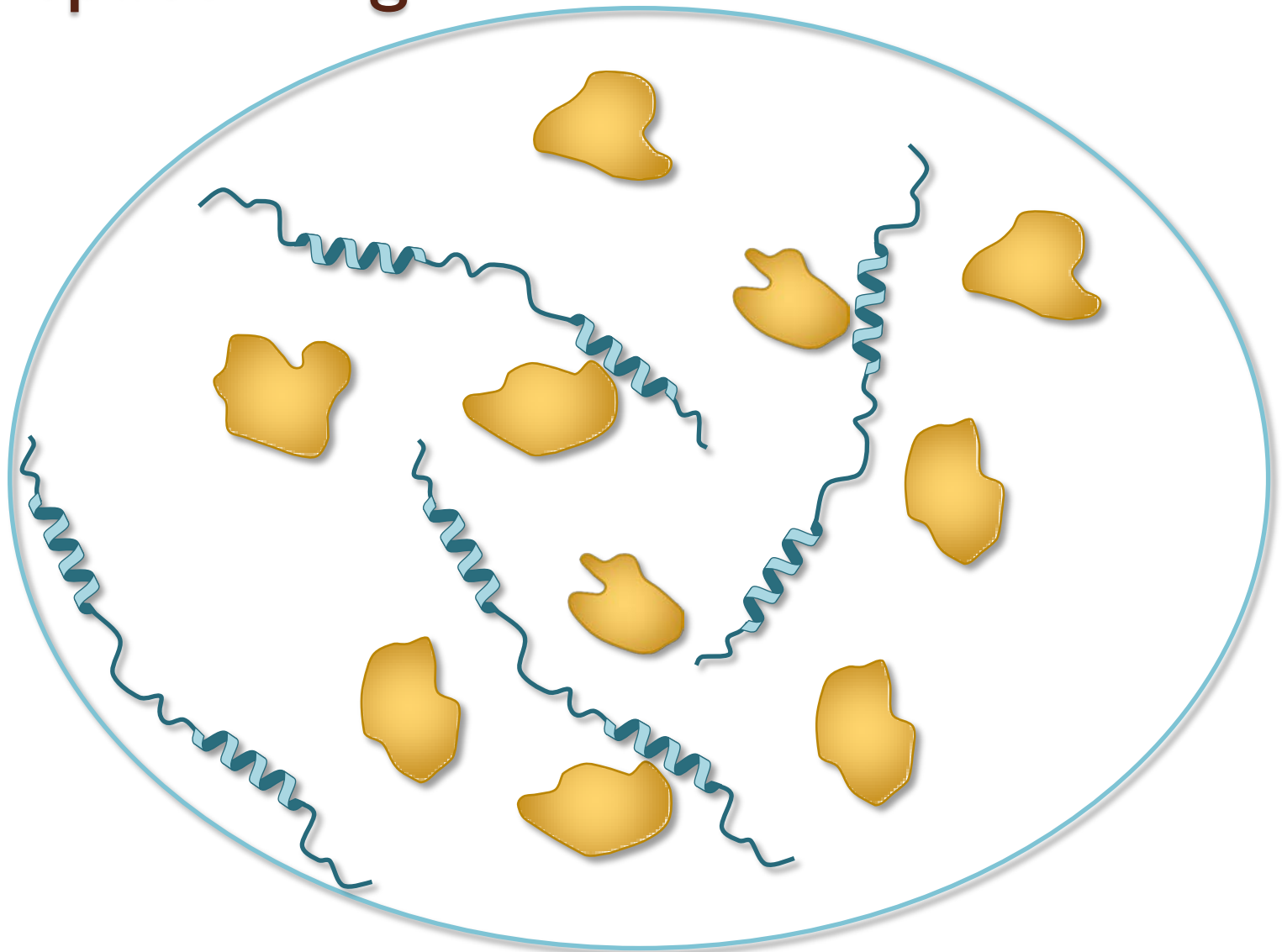


- Globular (GST) and disordered (Calpastatin inhibitory domain) control
- More than just space filling

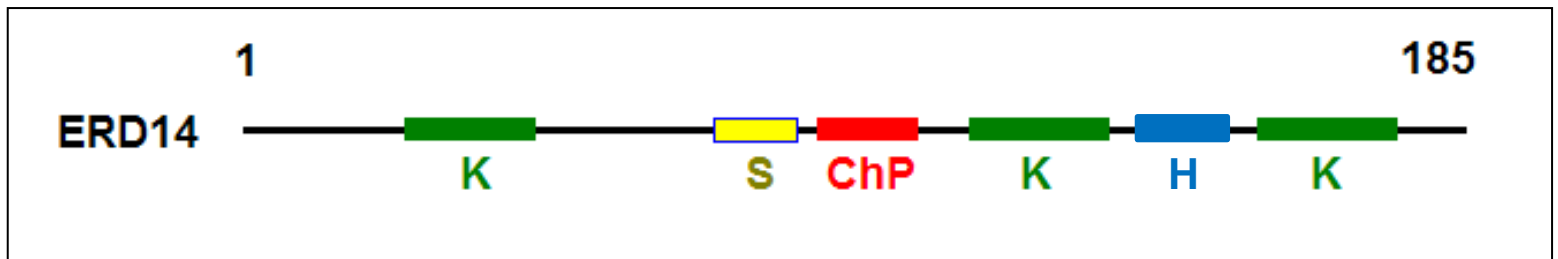
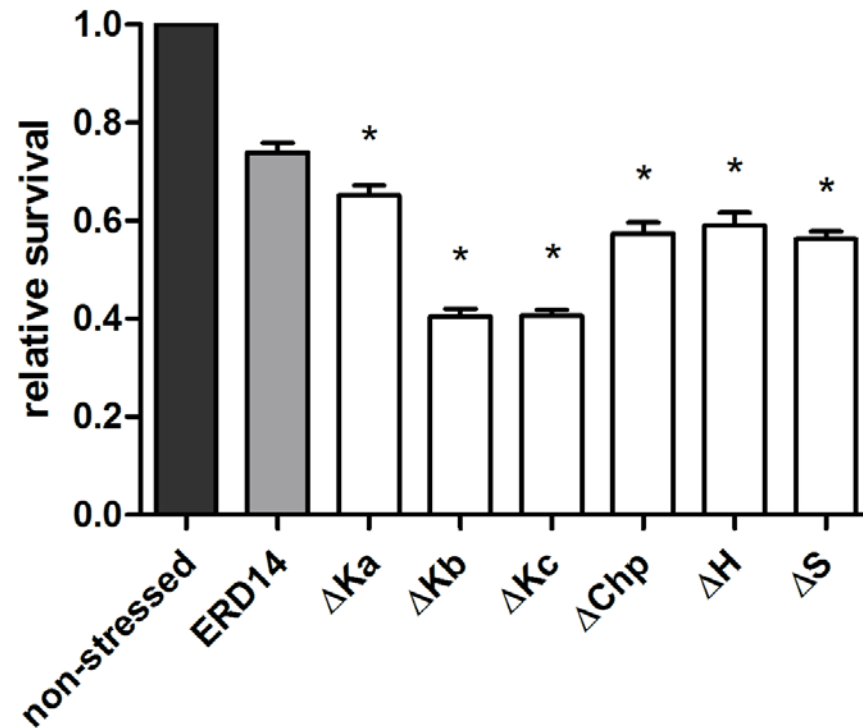
Theory II.: single binding and spacefilling



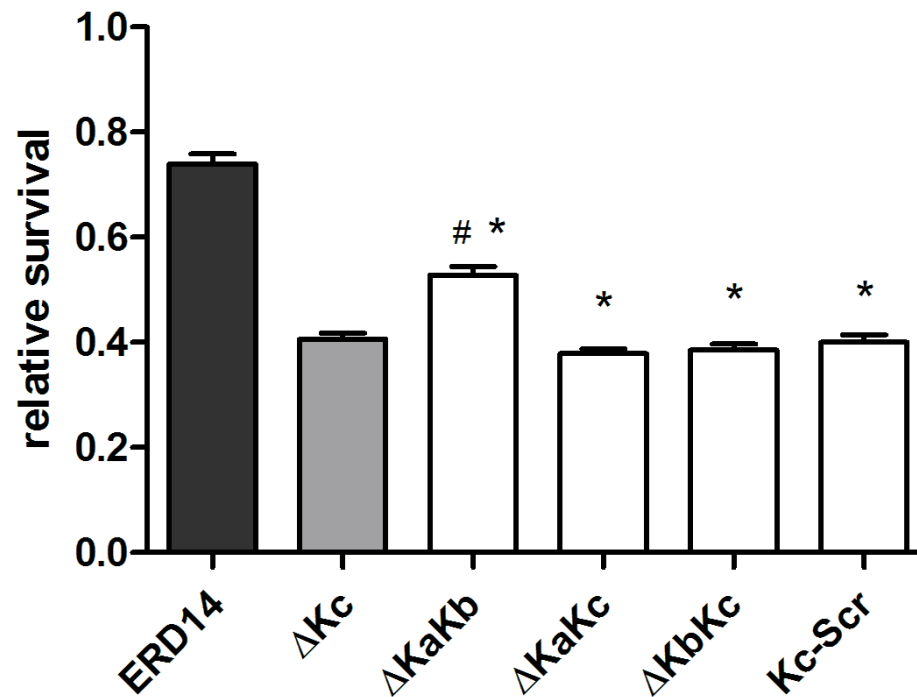
Theory II.: single binding and spacefilling



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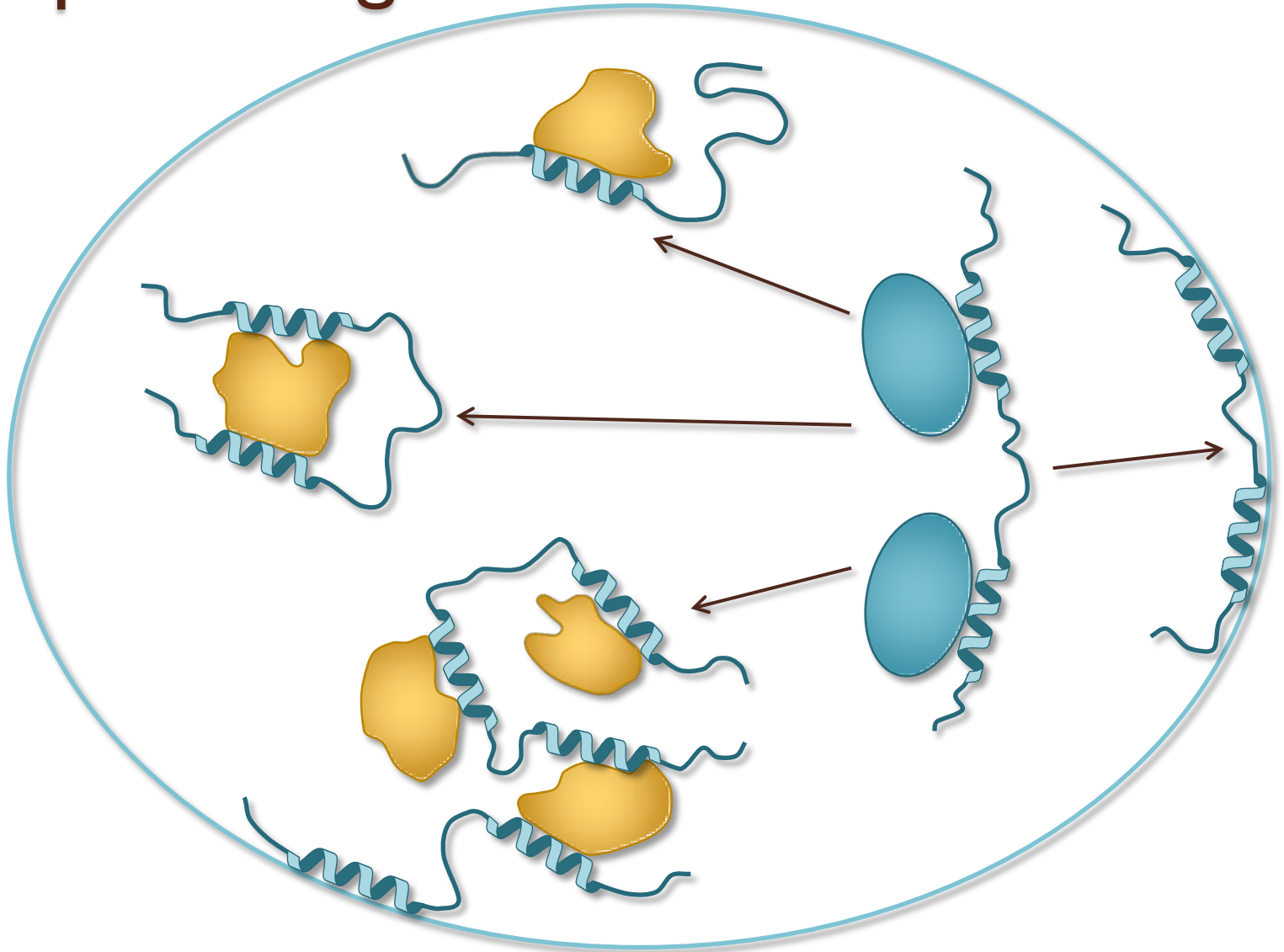


Theory II.: single binding and spacefilling

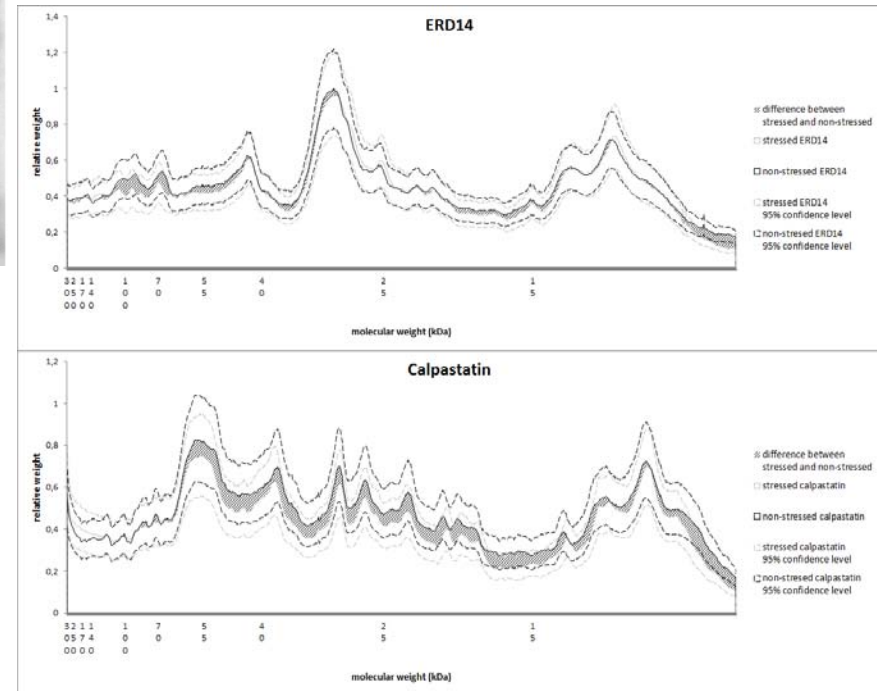
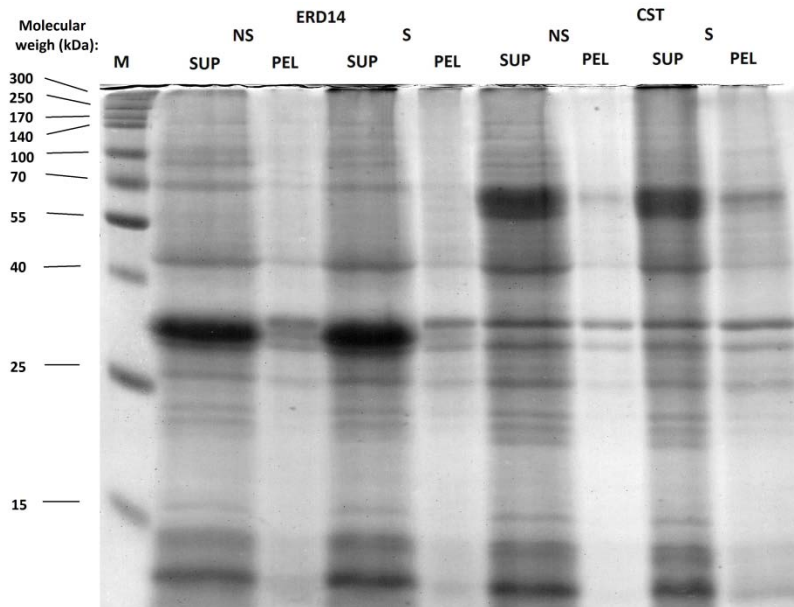


- One motif is not enough

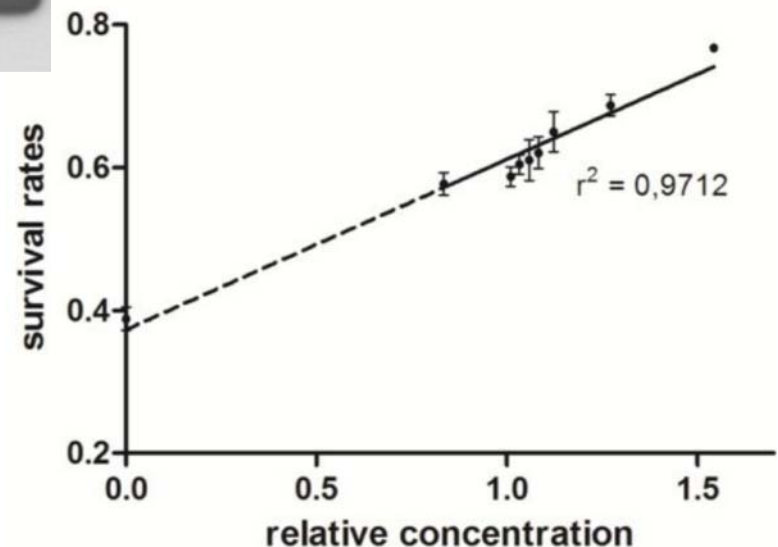
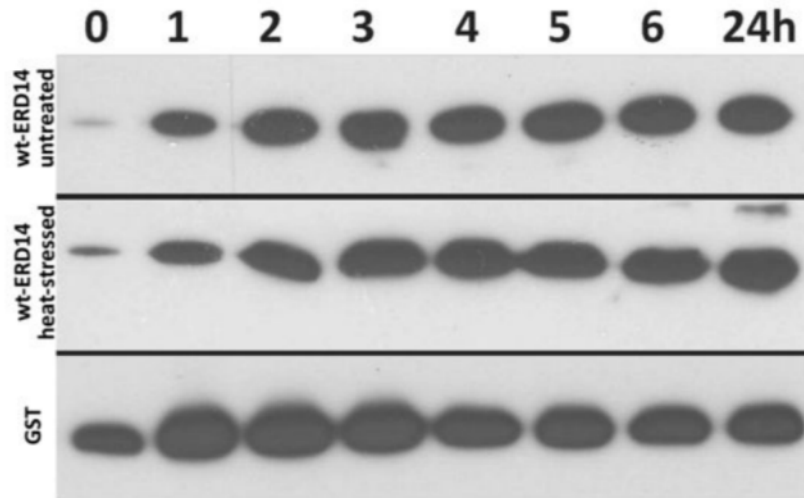
Theory III.: Multiple binding and spacefilling



Proteome protection



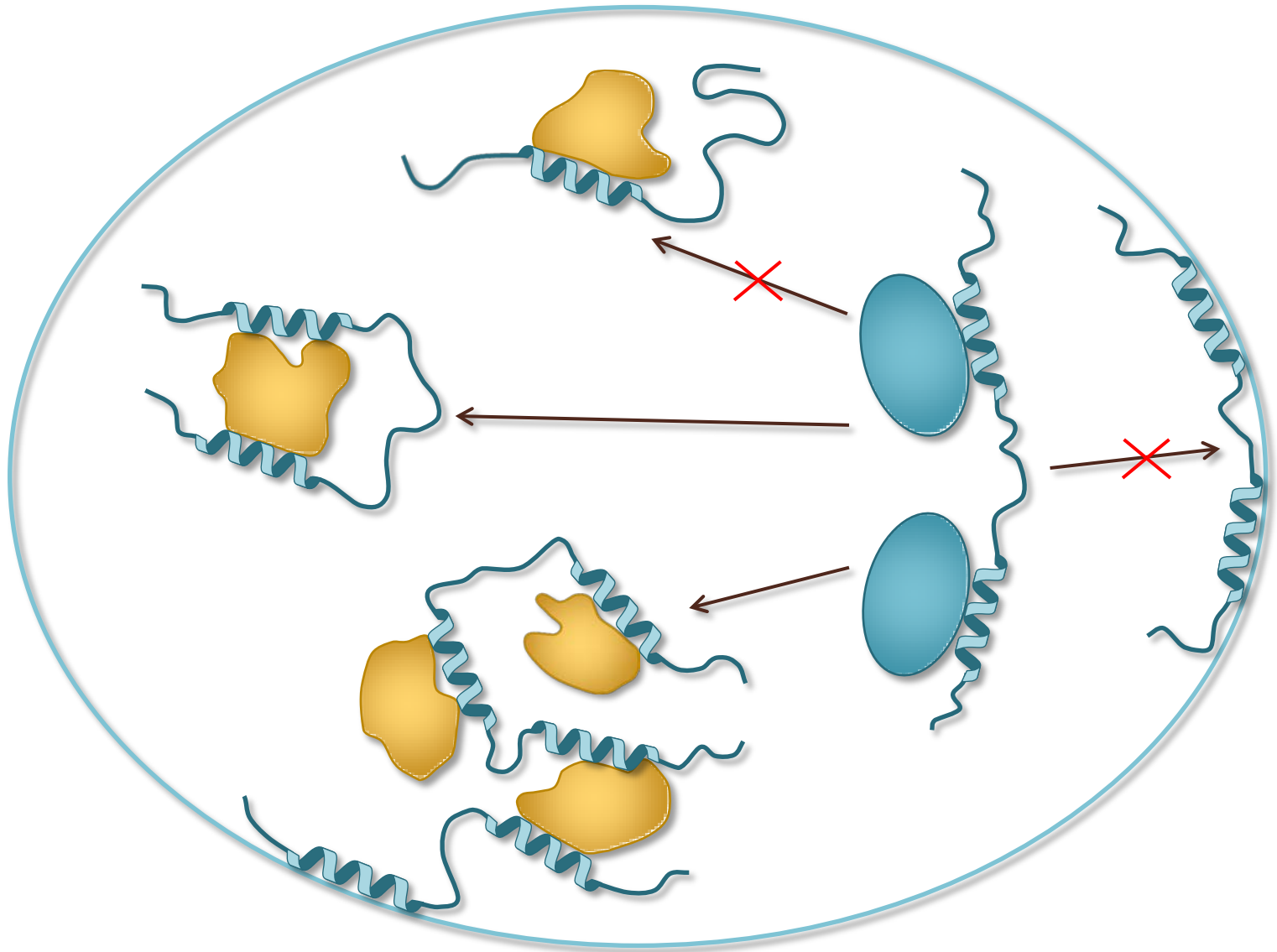
Half-time and concentration dependence



Summary

- ERD14 is disordered and has chaperone effect *in vitro*
- ERD14 is **disordered in E. coli** (in cell NMR) and have **cell defending effect (chaperone) *in vivo*** in the same cell with the same conditions
- No physiological partners in E. coli → **universal chaperone**
- Chaperone effect is related to **conserved elements** (motifs, PresMos)
- The primary partners are the proteins

Molecular mechanism



Complementary experiments

- Ultrafast in-cell NMR
 - Before stress
 - During stress
 - After stress
- Localization of the ERD14 protein during stress
 - Confocal microscopy
 - Electron microscopy

ERD group



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THANKY